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Conclusions: The Stratos Pro nebulizer and Europa compressor combination was evaluated with albuterol sulfate, ipratropium bromide, and budesonide which are standard, commercial inhalation formulations. Aerosol characteristics, including particle size, respirable/ fine particle fractions, nebulization time, output efficiency, and simulated inhaled mass were acceptable for effective pulmonary delivery of either albuterol sulfate and ipratropium bromide. Performance with budesonide microcrystalline suspension was less effective producing larger aerosol particle sizes, reduced percentages in the respirable range, and low levels of inhaled mass.

Results Certification:

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Figure 1: Testing set up configuration of the Stratos Pro nebulizer with Europa compressor with the NGI for EN 13544-1.

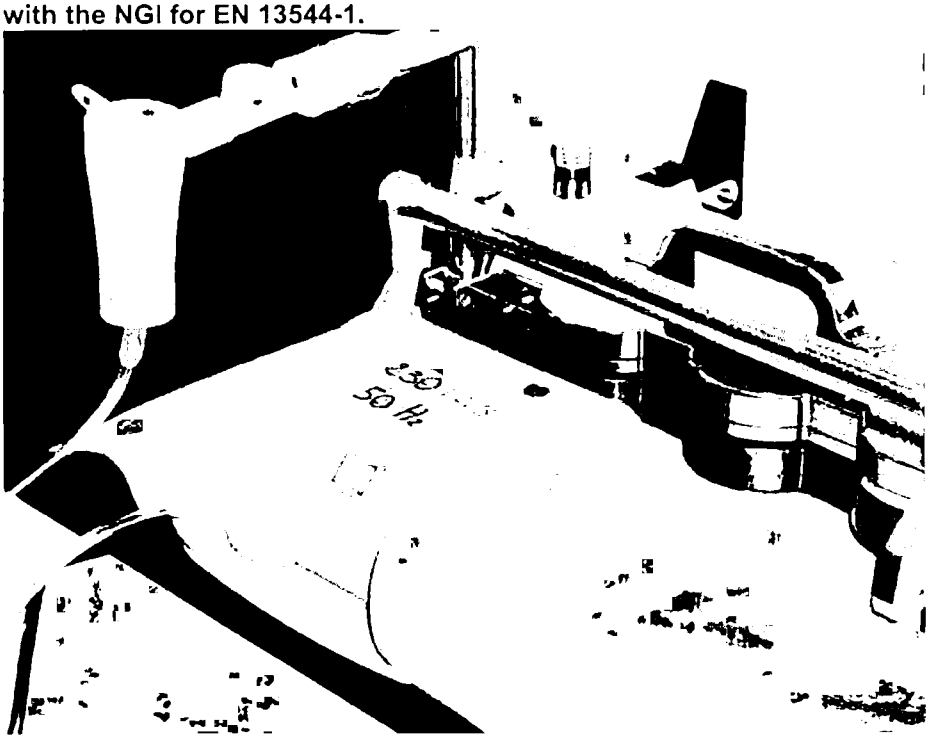

Figure 2: Testing set up configuration of the Stratos Pro nebulizer with Europa compressor for aerosol output and inhaled dose with EN 13544-1.

Table 9. Budesonide output test of Stratos Pro nebulizer and Europa Compressor.

Aerosol Output Test Parameter	Mean ± standard deviation
Budesonide Mass Loaded (test solution grams)	1.97±0.10
Budesonide Nebulized Mass Output (test solution grams)	1.18±0.04
Timed Output to Dryness (minutes)	2.44±0.33
Nebulized Output Efficiency (%)	59.6±0.58
Nebulized Flow Rate (ml/minute)	0.49±0.054
Budesonide Mass Inhaled (µg in filter; 15 bpm/ 500 ml V_T)	43.0±19.3
Temperature	23.46±0.714
Humidity	57.1±2.584

Budesonide Pulmicort

Table 7. Aerosol testing of Stratos Pro nebulizer and Europa Compressor with Budesonide.

Aerosol Parameters (NGI with budesonide)	Mean ± standard deviation
Mass median Aerodynamic Diameter (MMAD; µm)	5.77±0.68
Geometric Standard Deviation (GSD)	1.79±0.177
Respirable Fraction (% <5 µm)	40.1±8.6
Fine Particle Fraction (%<3.3 µm)	24.5±10.0
Temperature	23.0±1.0
Humidity	50.0±1.0

Table 8. Distribution of Budesonide in NGI induction port, collection cups, and micro-orifice collector (MOC) with Stratos Pro nebulizer and Europa Compressor.

NGI Stage/Size Range µm	Budesonide µg/stage (0-2 minutes; mean±sd)
Induction Port	2.08±1.81
1 (13.3-14.9)	6.86±2.26
2 (8.49-8.72)	9.33±3.27
3 (5.37-5.42)	19.23±9.79
4 (3.29-3.32)	23.0±10.1
5 (2.07-2.09)	13.29±2.52
6 (1.35-1.37)	3.9±1.92
7 (0.97-0.98)	0.49±0.85
MOC	0

Table 5. Distribution of Ipratropium in NGI induction port, collection cups, and micro-orifice collector (MOC) with Stratos Pro nebulizer and Europa Compressor.

NGI Stage/Size Range µm	Ipratropium µg/stage (0-2 minutes; mean±sd)	
Induction Port	7.57±5.7	
1 (13.3-14.9)	10.99±4.93	
2 (8.49-8.72)	15.7±5.72	
3 (5.37-5.42)	24.82±10.83	
4 (3.29-3.32)	32.80±9.12	
5 (2.07-2.09)	40.13±12.64	
6 (1.35-1.37)	23.17±3.64	
7 (0.97-0.98)	15.79±4.42	
MOC	9.5±3.78	

Table 6. Ipratropium Bromide output test of Stratos Pro nebulizer and Europa Compressor.

Aerosol Output Test Parameter	Mean ± standard deviation
Ipratropium Bromide Mass Loaded (test solution grams)	2.412±0.04
Ipratropium Bromide Nebulized Mass Output (test solution grams)	1.494±0.0924
Timed Output to Dryness (minutes)	4.06±0.41
Nebulized Output Efficiency (%)	61.93±4.06
Nebulized Flow Rate (ml/minute)	0.372±0.06
Ipratropium Mass Inhaled (µg in filter; 15 bpm/ 500 ml V_T)	76.27
Temperature	21.2±0.71
Humidity	50.8±1.33

Table 3. Aerosol output test of Stratos Pro nebulizer and Europa Compressor with Albuterol Sulfate.

Aerosol Output Test Parameter	Mean ± standard deviation
Albuterol Sulfate Mass Loaded (test solution grams)	2.96±0.015
Albuterol Sulfate Nebulized Mass Output (test solution grams)	2.03±0.11
Timed Output to Dryness (minutes)	4.12±0.42
Nebulized Output Efficiency (%)	68.63±3.51
Nebulized Flow Rate (ml/minute)	0.50±0.04
Albuterol Mass Inhaled (µg in filter; 15 bpm/ 500 ml V_T)	394.1±98.5
Temperature	23.3±0.06
Humidity	55.7±1.86

Ipratropium Bromide

Table 4. Aerosol testing of Stratos Pro nebulizer and Europa Compressor with Ipratropium Bromide.

Aerosol Parameters (NGI with ipratropium bromide)	Mean ±standard deviation
Mass median Aerodynamic Diameter (MMAD; µm)	4.0±0.46
Geometric Standard Deviation (GSD)	2.39±0.33
Respirable Fraction (% <5 µm)	60.9±7.61
Fine Particle Fraction (%<3.3 µm)	49.24±1.24
Temperature	22.43±0.32
Humidity	56.0±3.61

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Data

Stratos Pro nebulizer and Europa Compressor with commercial inhalation formulations.

Albuterol Sulfate

Table 1. Aerosol testing of Stratos Pro nebulizer and Europa Compressor with Albuterol Sulfate.

Aerosol Parameters (NGI with 0.083% albuterol sulfate)	Mean ±standard deviation
Mass median Aerodynamic Diameter (MMAD; µm)	3.79±0.18
Geometric Standard Deviation (GSD)	2.25±0.11
Respirable Fraction (% <5 µm)	64.19±3.82
Fine Particle Fraction (%<3.3 µm)	46.93±2.97
Temperature	22.6±0.52
Humidity	54.13±7.41

Table 2. Distribution of Albuterol in NGI induction port, collection cups, and micro-orifice collector (MOC) with Stratos Pro nebulizer and Europa Compressor.

NGI Stage/Size Range µm	Albuterol µg/stage (0-2 minutes; mean±sd)
Induction Port	4.08±7.1
1 (13.3-14.9)	30.14±5.4
2 (8.49-8.72)	43.11±8.52
3 (5.37-5.42)	84.16±9.36
4 (3.29-3.32)	103.71±9.37
5 (2.07-2.09)	107.29±12.08
6 (1.35-1.37)	70.92±2.31
7 (0.97-0.98)	39.8±1.39
MOC	15.14±1.21

Albuterol analysis by high pressure liquid chromatographic (HPLC). Albuterol concentrations were determined using a HPLC system equipped with WISP 712B autosampler, 501 pumps, 486 UV detector, and 746 Integrator (Waters Corp, Millford, Mass.). A Waters Nova-Pak C18 (3.9x150 mm) column at room temperature was used with peak detection at 276 nanometers. The mobile phase utilized for these studies was 50:50 methanol/water containing 1.43% acetic acid at a flow rate of 1.0 ml per minute. The assay sensitivity was 10 ng albuterol. Samples for HPLC analysis were dissolved directly into ultrapure water.

Ipratropium assay: A spectrophotometric method was used to measure the colorimetric reaction between ipratropium bromide and potassium permanganate reagent. Samples from the NGI or inhalation filters were initially dissolved in 3 ml methanol, evaporated to dryness, and then reconstituted with 1 ml of 50 nM NaOH. A standard curve was generated using diluted ipratropium in 50 mM NaOH and 50 µg/ml KMnO4. After 30 minutes reaction at room temperature, the optical density was read at 630 nm in a microplate reader. The sensitivity of the assay was 20 ng.

Budesonide analysis by HPLC. Budesonide concentrations were determined using a HPLC system on a Waters Nova-Pak C18 (3.9x150 mm) column at room temperature with peak detection at 238 nanometers. The mobile phase utilized was 50:50 ethanol/water at a flow rate of 0.8 ml per minute. The assay sensitivity was 10 ng. Samples for HPLC analysis were dissolved directly into methanol.

Results

The Stratos Pro with Europa Compressor was evaluated with standard commercial inhalation formulations of 0.083% albuterol sulfate inhalation solution (Dey; 2.5 mg/3 ml), 0.02% ipratropium bromide inhalation (Dey; 0.5 mg/2.5 ml), and budesonide inhalation suspension (Pulmicort Respules; 0.5 mg/2 ml).

With albuterol sulfate, the emitted aerosol particles were heterodisperse with MMAD of 3.79 µm and greater than 64% within the optimal range for inhalation (<5.0 µm) (Table 1). Approximately 47% of particles were in the fine particle range of <3.3 µm and capable of reaching into the peripheral lung tissues. Within the collection cups of the NGI, most of the albuterol was also distributed to stages 3,4, and 5 between ECD 2.08 and 5.39 µm (Table 2). By the aerosol output test, the Stratos Pro nebulized 69% of the charged 0.083% albuterol dose in 4.12 minutes at a flow rate of 0.5 ml/minute. Using the charge volume of 3 mls, the calculated inhaled albuterol dose was 394 µg (Table 3).

With ipratropium bromide, the emitted aerosol particles were heterodisperse with MMAD of 4.0 µm and 61% within the optimal range for inhalation (<5.0 µm) (Table 4). Approximately 49% of particles were in the fine particle range of <3.3 µm and capable of reaching into the peripheral lung tissues. Within the collection cups of the NGI, most of the albuterol was also distributed to stages 3,4, and 5 between ECD 2.08 and 5.39 µm (Table 5). By the aerosol output test, the Stratos Pro nebulized 62% of the charged 0.02% ipratropium bromide dose in 4.1 minutes at a flow rate of 0.37 ml/minute. Using the charge volume of 2.5 mls, the calculated inhaled ipratropium dose was 76 µg (Table 6).

With budesonide inhalation suspension, the emitted aerosol particles were heterodisperse with MMAD of 5.77 µm and 40% within the optimal range for inhalation (<5.0 µm) (Table 7). Approximately 25% of particles were in the fine particle range of <3.3 µm and capable of reaching into the peripheral lung tissues. Within the collection cups of the NGI, most of the budesonide was also distributed to stages 3,4, and 5 between ECD 2.08 and 5.39 µm (Table 8). By the aerosol output test, the Stratos Pro nebulized 60% of the charged budesonide dose in 2.44 minutes at a flow rate of 0.49 ml/minute. Using the charge volume of 2 mls, the calculated inhaled budesonide dose was 43 µg (Table 9).

February 6, 2007

Missouri Foundation for Medical Research: Final Report

Study Title: Aerosol Testing of Stratos Pro by the European Standard Method EN 13544.

Test Site: Pulmonary Research Laboratories, Department of Internal Medicine, Pulmonary Division, Missouri University School of Medicine and Harry S. Truman Memorial Veterans' Hospital, Columbia, Missouri

Testing Dates: 12/8/06 to 1/19/07

Test Devices: Stratos Pro nebulizer with Europa compressor.

Test Agents: Albuterol Sulfate (0.083%; 2.5 mg/3 ml), and Pulmicort Budesonide Inhalation Suspension (500 µg/ 2 ml respule).

Testing Protocol: European Standard Method (EN 13544-1)

Testing Procedures:

Aerosol particle size distribution by cascade impaction with the Next Generation Pharmaceutical Impactor (NGI): A Next Generation Pharmaceutical Impactor (MSP Corporation, Shoreview, MN) was used for aerodynamic particle sizing of pharmaceutical aerosols. Nebulizers were connected by an adapter/mouth piece attached to the induction port as demonstrated in Figure 1. With the Europa compressor, an Adaptive Power Systems FC100 converter was used to provide 230 volts/ 50 Hz. Aerosols were generated using a fill mass of the designated unit dose vial with the test devices nebulized under ambient laboratory conditions of room temperature (average 23°C) and humidity (average 50% RH) and collected for two minutes. Sampling flow rates were 15 L/minute for the NGI. Aerosolized drug particles were collected by impaction onto the collection cups of the NGI. The mass of nebulized albuterol sulfate (2.5 mg/3 ml), ipratropium bromide (500 µg/2.5 ml), and budesonide (500 µg/2 ml) was determined by spectrophotometric or HPLC analysis. An induction port was used to collect aerosol particles that did not penetrate into the impactors. After determination of the drug mass deposited in the induction port, on each stage/cup, micro-orifice collector, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosol was calculated on a log probability scale with the effective cutoff diameter as the ordinate and the cumulative percent less than the size range (by mass) as the abscissa (Kaleida Graph 3.51). The MMAD metrics were used as measure of central tendency. The respirable fraction (RF) and fine particle fraction (FPF) were calculated as the percentage of aerosolized albuterol at <5.0 and <3.3µm, respectively. Three determinations were performed for each test device.

Determination of Aerosol Output Rate and Inhaled Drug Mass. Aerosols were measured with a simulated human lung system using a valved, respirator pump (Harvard Apparatus, Dover, MA). Total aerosol output from the jet nebulizers was collected onto low resistance bacterial inhalation filters (Bemes Inc., Fenton, MO). Each device was operated to dryness (as assessed by visual endpoint where no aerosol plum could be visualized) with tidal volumes (V_T) of 500 ml at 15 breaths/minute in a sinusoidal pattern. The test device was weighed on an analytical balance and a fill mass of albuterol sulfate (2.5 mg/3 ml), ipratropium bromide (500 µg/2.5 ml), or budesonide (500 µg/2 ml) was added. The device was connected to the inhalation filter as demonstrated in Figures 2. The nebulizer was switched on and run to dryness and the time recorded. The end mass was determined and the output efficiency calculated by end mass/start mass x 100%. The deposited albuterol sulfate was extracted with ultrapure water and determined by HPLC assay. The deposited ipratropium bromide was extracted with absolute methanol, concentrated, and quantified by spectrophotometric assay. The deposited budesonide was extracted with methanol and determined by HPLC assay. The extraction efficiency from the filter was greater than 95 percent. Three determinations were performed for each test device.