Introduction

The concurrent delivery of the inhaled corticosteroid (ICS) fluticasone propionate (FP) and long-acting (62-adenosine bronchodilator (LABA) salmeterol xinafoate (SX)) is important medication to treat both the inflammatory and bronchoconstrictive elements of asthma (1). This superior control has been attributed to the mutual synergistic actions of the drugs when taken together (2), including the activation of the glucocorticoid receptor by salmeterol and its effects on other cellular targets related to inflammation and bronchoconstriction leading to lower doses and improved safety and adherence.

The addition of a LABA not only exerts salutary effects but improves lung function and symptom control to a greater extent than either component alone (4). The co-location of actives from a physical mixture is occur randomly when derived from the same stock. However, it is possible to produce products whereby the particles can be delivered in increased airspace relative to targeted areas of the lung. This approach can help achieve a more uniform delivery to the target cells and pathologies of inflammation and bronchoconstriction leading to lower doses and improved safety and adherence.

Methods and Materials

Particle preparation: The UMAX® (Ultrasound Medicated Amorphous to Crystalline translational particle engineering technique) (5) can be used to manufacture highly stable and high surface area spherical particles for optimal delivery to the lung (6). Particles of 5.1 mass ratio FP/SX (Figure 1) were prepared using acetone solvent for the initial spray drying of the UMAX process. The spray dried particles were ultrasonically treated in a non-solvent (perfluorodecalin) prior to isolation.

Figure 1: Scanning electron micrograph and Symptomatic particle size distribution of combination UMAX particles of FP and SX with v = 4.47 μm

Formulation and aerosolization: The aerodynamic efficiency of the combination particles was evaluated in DPI formulations (2.5% w/v with ML03 lactose). The blend strength of each formulation equaled to 250 μg FP and 50 μg SX (equivalent to Advair 250/50). Determination of the constant formulation and analysis of samples from the Next Generation Impactor (NGI) were carried out using HPLC (2,7).

The drug content uniformity of the blended formulation was assessed (8), targeting a relative standard deviation (RSD) of individual batches less than 2.5%. Using gaschromatography/mass spectrometry (GCMS) capsules at a mass of 0.1 mg powder blend, aerodynamic assessment for both the UMAX particle blend and a commercially available Advair 250/50 device (ejected into a capsule) was carried out using the NGI using both the Rotahaler and Cyclohaler devices, discharging with air flows of 60 L/min for 4 s and 90 L/min for 2.8 s respectively.

The mass of drug deposited on each part of the NGI was determined by HPLC and the mass median aerodynamic diameter (MMAD, geometric standard deviation (GSD), fine particle dose (FPD) - stages 3 - 4 of the NGI) and fine particle fraction of the loaded dose (FPFD) were determined.

Conflon Raman spectroscopy: The engineered particles were characterized by Confocal Raman spectroscopy to study the relative distribution of crystalline phases of each active ingredient.

NGI Impactor Testing: Stability of DPI formulation

The stability of dry powder formulations is an important consideration for respiratory drug formulations given the need for consistent and reproducible performance over the lifetime of an inhalation device. The aerosol performance of FP and SX multi-component UMAX particles was assessed using a Rotahaler device at an initial time-point and following 6 month storage at 25 °C/75% RH (Figure 2). Consistent deposition profiles were seen for both FP and SX indicating maintenance of co-deposition of both FP and SX on stage 2 - 4 of the NGI irrespective of time between formulation and actual impactor testing.

Figure 2: Stage by stage deposition of FP and SX aerosolized from a Rotahaler containing UMAX FP/SX multi-component particles at initial formulation and after 6 months storage

NGI Impactor Testing: Consistent Delivery of Both Actives

The aerosol dispersion performance and uniformity of delivery across the stages of impactor were evaluated in order to provide an indication of the potential benefits of processing the actives into a single particle compared with dry powder blends of mixed APIs. The blend content uniformity was found to be very high as evidenced by the very low RSD of 2.5% with respect to both drugs when 10 random samples of 12.5 mg (equivalent to one unit dose) were analyzed by HPLC. While the data suggested that the dose delivered from the formulations, following aerosolization from a Rotahaler or Cyclohaler, were consistent with the formulated dose, they did not indicate uniform dose delivery of both actives over all the impactor stages.

Figure 3: Comparison of mass deposited on each stage of the NGI for Advair 250/50, UMAX particles and non-optimized UMAX particles

The data shows that for optimized UMAX particles, both actives were delivered consistently together across stages 2 - 4 (Figure 3). The normalized mass ratios of FP/SX were found to be consistent with the formulated mass ratio. In contrast, the mean mass ratio of FP/SX deposited following the aerosolization of the Advair formulation from a Rotahaler and Cyclohaler on the same stages was approximately higher, which suggested inconsistent delivery of FP and SX to the lower stages of the NGI with approximately 15% greater FP delivery than SX. Non-optimized UMAX particles with a change in morphology from spherical to plates during processing performed very poorly.

Figure 4: Chemical structures of fluticasone propionate (FP) - left, and salmeterol xinafoate (SX) - right

Raman Laser Spectroscopy

Raman laser spectroscopy is a non-destructive technique for identifying individual drug particles by acquisition of chemical information about the molecules (4) involved in the Raman scattering process. Each crystalline structure contains one or more Raman active, i.e., first order infrared active vibrational modes that can be determined (5) which are then used to generate Raman images using component analysis. An image is derived from several thousand spectra and is computed by integrating over a specific reference Raman in the spectra. The intensity calculated from each spectrum is colour coded. Once each individual material has been analysed, multicomponent particles or mixtures of powdered particles can be analysed.

Figure 5: Reference Raman spectra for FP and SX collected from several regions of the pure FP and SX species using 532 nm laser wavelength

Conclusions

The spectra of FP and SX showed distinct peaks at 1690 cm⁻¹ and 1350 – 1480 cm⁻¹ respectively and were used to detect crystalline phases of each component on the microscope image. The Raman microscope has been used to analyse mixed API powders to understand the distribution and chemical species present. The analysis indicated that the material was extremely well mixed as shown by the yellow areas on the Raman image which reveal the presence of both crystalline species within the same particle (Figure 6).

Figure 6: Left - Raman image with colour mapping of UMAX particles on stage 4 of the NGI and Right - Raman image with colour mapping of Advair 250/50 particles on stage 4 of the NGI, in which the particles containing SX are coloured red and pixels containing both FP and SX are coloured yellow.

The deposition of FP and SX on stage 4 of the NGI following aerosolization of Advair 250/50 and combination UMAX particles from a Cyclohaler was assessed using confocal Raman spectroscopy. The reference spectra were used to generate Raman images and thus the location of the drug components in the field of view. The Raman analysis of the sample indicated that each particle contained both crystalline actives as shown by the dominance of yellow colour (Figure 6 - left); noteworthy, this sample was obtained from a liquefied DPI blend with only 2.5% actives, and it is possible the lactose may have interacted with actives during Raman analysis. However, for fair comparison, the Advair 250/50 particles when subject to identical analysis showed no co-association of the FP and SX particles as evidenced by the total lack of yellow colouration (Figure 6 -right).

Figure 7: Raman image with colour mapping (image area 388.4 μm × 237.6 μm (Y) of UMAX particles in which the particles containing FP are coloured red, the pixels containing SX are coloured green and pixels containing both FP and SX are coloured yellow.

The dry powder combination particles (100 % active and no lactose) were also analysed prior to impactor testing. The analysis indicated very good homogeneity and distribution of both crystalline actives in a very high proportion of the actual particles as shown by the dominance of yellow colouration (Figure 7), but with some noticeable and distinct crystalline-phases of both FP and SX.

Confocal Raman Spectroscopy

Ultrafast laser engineering techniques can be used to manufacture microcrystalline dual component particles of an ICS and a LABA for both DPI and NDI with high homogeneity. These particles should facilitate optimal and consistent delivery of both drugs to the lung. The UMAX combination formulation process used for manufacture combination particles circumvents the use of mechanical milling and subsequent variable performance.

The particles can have optimal performance attributes for inhalation products designed for asthma and COPD. Particle of both FP/SX are well suited for DPI, NDI or aerosol delivery, with a non-destructive UPPS, HPLC formulation, and Raman microscopy to identify individual drug particles co-deposited within the impactor. Comparative studies have been carried out using the Advair / Serevent combination at 250/50 mass ratio of FP and salmeterol.

The combination particle formulations consistently deliver both active ingredient to each stage of the impactor and have shown data for several months. Raman microscopy images provide a new and useful tool to identify individual particles are intimately co-associated which should facilitate improved and consistent delivery of each active ingredient. This is in contrast to the Advair / Serevent formulation which showed no co-association by confocal Raman spectroscopy and inconsistency of delivery of both actives to the fine particle stages of the NGI.

Inhaled combination therapy is an exciting area of research in order to develop improved respiratory medicines and potentially offers patients the best medication options for pulmonary disease. Co-formulated drugs will not only improve any synergy of action but may yield opportunities for dose sparing when designing near ICS / LABA structure drug products for respiratory disease.

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