Assessment of Drugs and Carrier PSD in a Commercial Combinational Dry Powder Inhaler Post Multistage Cascade Impaction Analysis by Raman Imaging

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SUMMARY

Multistage cascade impaction is the accepted analytical method for investigating the aerodynamic particle size of Dry Powder Inhalers (DPI). In addition to the cascade impaction, FDA recommends microscopic evaluation of the sample for "release and stability purposes" to check for the presence of large particles, aggregates and foreign contaminants, changes in morphology and crystal growth for both drug substance and carrier particles even though it is very subjective and not robust testing method.

In this study, Raman Chemical Imaging (RCI) was used to investigate the Active Pharmaceutical Ingredients (API) particle size distribution (PSD), carrier (Lactose) PSD and access qualitatively degree of API-carrier aggregation in a commercial DPI, ADVAIR DISCUS® 500/50 post multistage cascade impaction analysis.

As data suggest, there is a good correlation of both drug and carrier PSD as it related to the cut-off diameter of stage 3 and 4 of the cascade impactor from where DPI was sampled for Ingredient-specific Particle Sizing (ISPS) analysis by RCI. Assessment of free-standing API particles and bound to Lactose API particles was done on the fusion brightfield and RCI images by visual inspection of all detected API particles as it relates to the spatial location of all detected Lactose particles on a fusion image. 30% increase in the percentage of agglomerated with lactose API particles was detected on Stage 4 as compared to Stage 3.

INTRODUCTION

The structure of dry powder inhaler (DPI) formulations affects stability and drug dosage, owing to the complex surface interfacial forces between components of the formulation. The inhaled portion may contain the drug or the drug substance within a matrix of excipients-adhered to the carrier/other drug particle, which maybe related to their surface properties. PSD, particle morphology, degree of aggregates and moisture content can greatly influence the bulk properties of the formulation and the required product performance. Maintaining reproducibility in Inhaler Aerodynamic Particle Size Distribution (APSD) is a formidable challenge since it may be affected by interactions between API and excipient throughout the device's lifetime.

Multistage cascade impaction, even though accepted by FDA method, requires strict humidity control during inhaled particles aerodynamic size measurements to minimize hygroscopic growth and aggregation of particles and does not provide efficient means to analyze degree of aggregates in the formulation or to accurately estimate free-standing drug particles from bound to carrier drug particles.

Recent research on comparison of delivery characteristic from a combinational metered dose inhaler using Andersen Cascade impactor and Next Generation Pharmaceutical impactor have suggested challenges in assuring good repeatability and reproducibility of inhalation drug testing when these methods are used for drug PSD and dose delivery profiles analysis due to variations caused by manual actuations (operator errors), flow rates, induction ports [1]. Since the most critical attributes for DPI is reproducibility of the drug dose and drug PSD, microscopic evaluation of the both drug substance and carrier particles is performed for release and stability studies, as recommended by FDA [2], even though an experienced microscopist may fail in differentiating between multiple APIs or their different polymorphic forms and API-carrier aggregates. Therefore, accurate assessment of each ingredient PSD in the combination drug product is a challenging task to be addressed by microscopy or cascade impaction analysis alone.

RCI is a novel technology for ingredient-specific PSD determination. RCI obtains spectrally and spatially resolved images of a sample area that allows recognition of different chemical species as well as measurement of particle size. In most cases, RCI can easily separate API from other components in the formulated drug for ISPS analysis and for estimate of degree of aggregates. Spectral shifts as small as 2 cm^{-1} can be detected; therefore, RCI can determine polymorphic and hydrated forms of a pharmaceutical. RCI has been successfully applied for ISPS analysis of several corticosteroid aqueous suspensions (Rhinocort AQ™, Flonase™) and MDI (Combivent®) [3, 4]. Specifications for the Active Ingredients in DPI that can be obtained by RCI in addition to PSD is particle morphology, solvates and hydrates, clathrates, crystalline forms, polymorphs, amorphous forms, co-crystals, etc.

In this study, assessment of drugs and carrier PSD in a commercial combinational dry powder inhaler, ADVAIR DISCUS® 500/50 was used by Raman Chemical Imaging. ADVAIR DISCUS® contains both an anti-inflammatory corticosteroid (fluticasone propionate) and a long-acting bronchodilator (salmeterol xinafoate) working together to maintain breathing function. Additionally, lactose is added to the formulation as a bulking agent.

EXPERIMENTAL

ADVAIR DISCUS® 500/50 (GlaxoSmithKline, UK), was utilized in this study. Aluminum inserts were placed on stage 3 and 4 of the NGI, on to which aerosolized particles from the ADVAIR product were collected. A small portion of DPI powder from each plate was carefully transferred onto an Al-coated microscope slide and smeared evenly by using glass coverslip prior Raman Imaging measurements.

A Raman spectral library of all pure component materials was collected on a ChemImage Falcon II™ RCI System.

Figure 1 shows a plot of the pure component spectra over the full Raman spectral range (400 – 3200 cm^{-1}). In the RCI spectral region (1350-1690 cm^{-1}), Fluticasone propionate may be spectroscopically identified and separated from Salmeterol and lactose by the Raman peak at 1670 cm^{-1} whereas Salmeterol maybe be identified by the Raman feature at 1410 cm^{-1}.

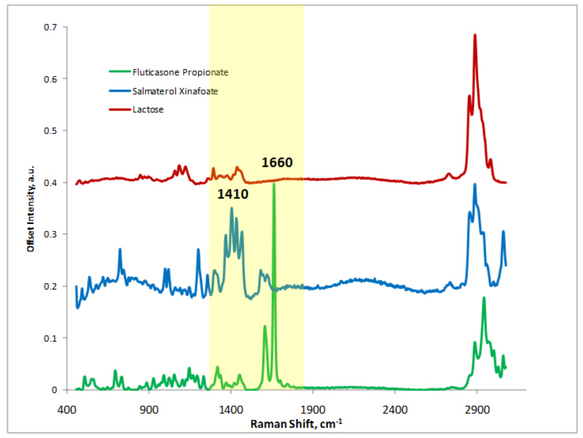

Figure 1. Raman dispersive spectra of dry powder pure components where the RCI spectral range has been highlighted in yellow.

RCI data was collected over a portion of the fingerprint spectroscopic region from 1350-1690 cm^{-1} which encapsulates the distinct Fluticasone propionate band observed from the Raman dispersive spectroscopy library development using a ChemImage Falcon II™. 100 Fields of View (FOV) were measured for DPI sample from each stage to achieve a minimum of 100 Fluticasone propionate particles, resulting in 0.2mm^2 area measured for each dry powder sample. A final API-specific particle image was analyzed for particle number and size* based on Equivalent Circular Diameter, the diameter of a circle that would have the equivalent area as the particle.

Sample	Stage	Particles	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
ADVAIR Discus® 500/50	S3	123	1.2	2.1	3.3	0.8	0.3	5.6
ADVAIR Discus® 500/50	S4	148	1.0	1.7	2.9	0.8	0.3	4.5

Table 1. Fluticasone propionate number-wise (not volume weighted) PSD statistics in dry powder sample based on Equivalent Circular Diameter.

Sample	Stage	Particles	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
ADVAIR Discus® 500/50	S3	49	1.0	1.6	3.4	0.9	0.5	4.6
ADVAIR Discus® 500/50	S4	55	0.9	1.6	2.7	0.7	0.3	3.4

Table 2. Lactose number-wise (not volume weighted) PSD statistics in dry powder sample based on Equivalent Circular Diameter.

** Based on Rayleigh criterion, the theoretical optical resolving power of the Falcon II for Raman Images obtained with 532 nm excitation light and 100x magnification (NA=0.95) is approximately 350 nm and multiple pixel constraint (3 pixels) is applied for the detection of a particle. The limit of detection by Falcon II RCI system and linearity of particle sizing was established for the system in the independent validation study (results now shown) based on NIST-traceable polystyrene (PSMS) sizing standards of sizes between 0.5 and 10 µm. It was determined that Falcon II is able to detect sub-micron (0.5 µm) PSMS particles.*

Table 1 and Table 2 summarize the Equivalent Circular Diameter size number-wise (not volume weighted) distributions for the measured samples, and the corresponding histograms are shown in Figure 2. As data suggest, there is a good correlation between aerodynamic particle size of the inhaled particles and the cut-off diameter of stage 3 and 4 of the cascade impactor.

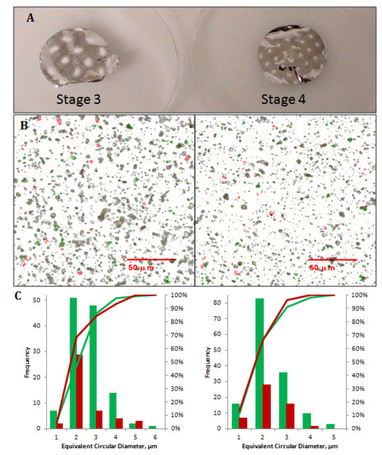

Figure 2. ADVAIR samples used for ISPS analysis: from stage 3 and 4 (A), Representative BFR/RCI Montage of ADVAIR samples from each stage where Fluticasone is false-colored in Green and Lactose is false-colored in Red (B); Fluticasone propionate Equivalent Circular Diameter histogram and cumulative percentage histograms for each sample where Fluticasone is false-colored in Green and Lactose is false-colored in Red (C).

Each FOV containing an API or carrier particle as confirmed by RCI was visually compared with an optical microscopy, Raman White Light and Raman Chemical Fusion image post ISPS analysis. If the identified API was determined to be an API-API or API-carrier agglomerate based on visual inspection, the nature of agglomerate was identified. Sizing of each identified agglomerate was performed automatically by ISPS algorithms, which operates on each detected aggregate defined by it's unique coordinates in the FOV.

Figure 3. Representative fusion BFR/RCI image of free standing and agglomerated particles in a DPI formulation where Fluticasone is false-colored in Green, Salmeterol is false-colored in Blue and Lactose is false-colored in Red.

Assessment of free-standing API particles (Figure 3, A) and bound to lactose API particles (Figure 3, C, D, E) was performed on the fusion BFR/RCI images by identifying co-localized detected Fluticasone particles (false colored in green on a fusion image, Figure 3), Salmeterol particles (false colored in blue on a fusion image, Figure 3) and Lactose particles (false colored in red on a fusion image, Figure 3). The percentage of free-standing API particles on Stage 3 was determined to be 90% whereas much more API-Lactose agglomerates were detected on Stage 4, and percentage of free-standing API was determined to be 60% out of all detected API particles. The size of agglomerates may be estimated by the D90 values and Maximum size of the particles detected from each stage of the cascade impactor and was shown to be correlated for D90 values with cut-off ranges of the stages 3 and 4 from where DPI was sampled for analysis. As shown on Tables 1 and 2, the largest drug particles detected from Stage 3 and 4 were of sizes 5.6 and 4.5 µm respectively, and the largest carrier particles detected from Stage 3 and 4, respectively were of sizes 4.6 and 3.4 µm.

CONCLUSIONS

Due to the critical impact of agglomerates in DPI formulation on dissolution profile and permeability of the drug within the sites of action (i.e. patient's lungs), accurate assessment of degree of free-standing versus aggregated drug particles is critical. RCI is shown in current study to have a potential to address the need in accurately identifying aggregates and sizing them based on spectroscopical identification. Imaging data collection and automated image processing sizing algorithms[5] can be challenged and validated on the model systems to show robustness and reproducibility. ISPS by RCI can be applied to study similarities in formulations tested for bioequivalence especially when currently available analytical methods fail to determine extend of agglomerates in test and reference products with sufficient precision and accuracy.

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