Particle Interactions of Fluticasone Propionate and Salmeterol Xinafoate in Advair Diskus®

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Summary

Particle co-associations between the active pharmaceutical ingredients (APIs) fluticasone propionate (FP) and salmeterol xinafoate (SX) were examined in a dry powder inhaled (DPI) combination product. Single Particle Aerosol Mass Spectrometry (SPAMS) was used to investigate the particle interactions in Advair Diskus® (500 mcg FP/50 mcg SX). A simple rules tree was used to identify each compound, either as a single API or co-associated at the level of the individual particle, using unique marker peaks in the mass spectra for the identification of each drug. High levels of API co-association were observed in the aerosols emitted from Advair Diskus®. The majority of the detected SX particles were found to be in co-association with FP in the tested device. Another significant finding was that rather coarse FP particles and fine SX particles were forming the particle co-associations in the DPI product.

Introduction

Two classes of drugs widely used in the treatment of pulmonary diseases are long acting β2-agonists (LABA) and inhaled corticosteroids (ICS). Clinically, prescribing both classes of active pharmaceutical ingredient (API) has become a method of taking advantage of their complementarity. There is significant clinical and in-vitro evidence to suggest that the co-association of ICS and LABA at the level of the single administered aerosol particle leads to a synergistic effect with improved clinical outcomes. Studies have shown increasing evidence of complementary and synergistic effects of LABA and ICS, interacting at the molecular receptor [1] and cellular [2] level. One postulated reason for this synergistic increase in pharmacological efficacy is the co-association of APIs at the point of deposition within the lung [3]. Specifically, salmeterol xinafoate (SX) was observed to retard the transport of fluticasone propionate (FP) across cell cultures of lung epithelial tissues, prolonging its localized anti-inflammatory effects [3]. The co-administration of the two APIs is expected to improve the likelihood of the two APIs being delivered to the same location in adequate concentrations, maximizing the likelihood of these synergistic effects [4]. Previously, it was demonstrated that there is some affinity between FP and SX in solution, indicating that they may also be co-associated at the level of the individual administered aerosol particle [5]. Attempts have been made to observe particle co-associations with Raman microscopy by Theophilus et al [6]. Determining the chemical composition of individual particles with a technique such as single particle aerosol mass spectrometry (SPAMS) should yield more definitive data. Preliminary runs with the SPAMS technique have already shown promising results [7].

The objective of these experiments was to determine the degree of particle co-associations between FP and SX across multiple size ranges in real-time. Axotide Diskus® (500 mcg) and Serevent Diskus® (50 mcg), each a single API product containing FP and SX, respectively, were used to identify unique marker peaks from mass spectral signals of each API.

Materials and Methods

The experimental setup of the SPAMS instrument (SPAMS 3.0; Livermore Instruments Inc.) for inhalational device testing is similar to that described by Morrical et al [7]. For the calibration of the SPAMS instrument in the region of 0.1-10 µm, polystyrene (PLS) microspheres (Thermo-Fisher Scientific) were used. Diskus® inhaler devices were fitted to an universal induction port (MSP Corp., Minnesota, USA) which was fitted to a pre-separator (Copley Scientific, UK) that was filled with 15 mL of water and then connected to a 4 L relaxation chamber. This assembly of pre-separator and relaxation chamber was then fitted to the SPAMS inlet. The primary purpose of the pre-separator and relaxation chamber was to dilute the sample and filter out coarse lactose carrier particles (>10 µm) to prevent clogging of the SPAMS inlet interface. The actuation of the DPIs was made with a 2.7 seconds draw of air into the relaxation chamber, at a flow rate of 90 L/min (i.e. 4 L of air drawn). Approximately the same number of particles was analyzed for each sample run (ca. 10,000 individual particles each). The desorption/ionization laser was set to fire at a wavelength at 248 nm. In the configuration being operated, the SPAMS was able to analyze up to 64 particles per second (laser repetition rate 64 Hz). The laser energy was maintained at approximately 12 mJ per pulse to obtain a high and consistent hit rate.

Results and Discussion

Figures 1-3 show the averaged mass spectra acquired from a single dose each of Axotide Diskus®, Serevent Diskus® and Advair Diskus® acquired from each single actuation. Figures 1 and 2 show spectra of pure FP and SX respectively, while Figure 3 shows spectra of co-associated particles of FP and SX.

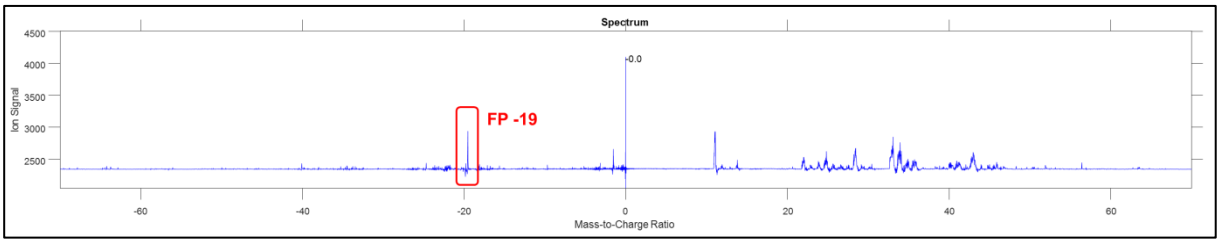

Figure 1 Averaged mass spectra of pure FP particles (M/Z = -19) from Axotide® Diskus®.

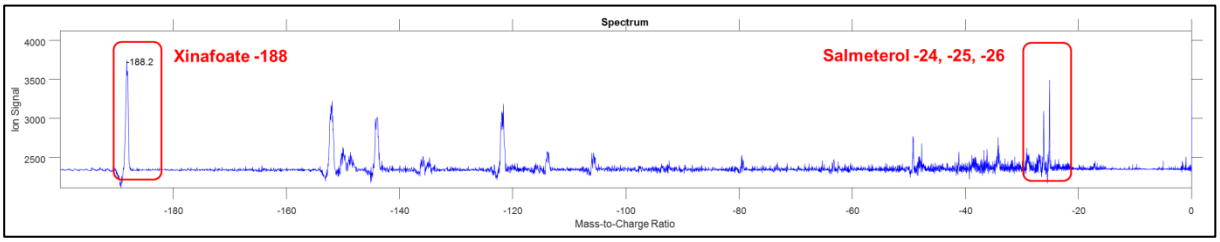

Figure 2 Averaged mass spectra for pure SX particles (M/Z = -188; -24, -25, -26) from Serevent® Diskus®.

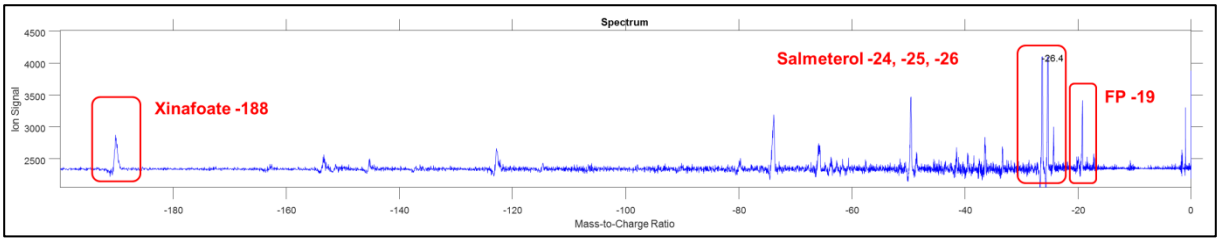

Figure 3 Mass spectra for co-associated particles of FP (M/Z = -19) and SX (M/Z = -188; -24, -25, -26) from Advair® Diskus®.

The mass spectra were individually evaluated for the presence of unique marker peaks that are indicated in Figures 1-3, with optimal predictive value for the identity of each API. The marker peaks inferred from the two products containing a single API were then used to discern those APIs in the combination product. Negative ions were primarily used as markers since unique peaks were more readily identified there. It was determined that a peak at M/Z = -19 was found in FP, indicating the liberation of a fluorine atom from the fluticasone structure. For SX, peaks at M/Z = -188 and -24, -25, -26 were identified as originating for the xinafoate counter ion (M/Z= -188) and salmeterol (hydrocarbons: C_2, C_2H, C_2H_2), respectively. It can be clearly seen that the spectra in Figure 3 contain marker ions that correspond to those seen in the spectra taken from pure standards of both FP and SX and must therefore be an agglomerate containing both types of API particles.

The plot in Figure 4 illustrates the aerodynamic particle size distribution (APSD) of APIs in the Advair Diskus®, either as single API particles or as co-associated API particles. The collected data demonstrated that there are a high number of co-associated particles emitted from the Advair Diskus®. Only a small fraction of particles could not be assigned probably due to insufficient ionization (5%). 41% of all the particles contained FP and SX in co-association (Table 1). Due to the far higher dosage strength of FP compared to SX in Advair Diskus® (500/50 mcg), there are many more FP particles present and detected than SX particles. A large fraction of SX (74% of total SX) was determined to be in co-association with FP. While only about 52% of FP was co-associated. This could be a simple result of the statistical mechanics given the 10x difference in dosage strength between the APIs in the product. The cohesive nature of FP [8] could plausibly also play a role for this occurred effect. At a sufficiently high FP to SX ratio, all available SX particles are used up in co-association and there is an excess of FP particles given the far higher dosage strength of FP in the analyzed formulations.

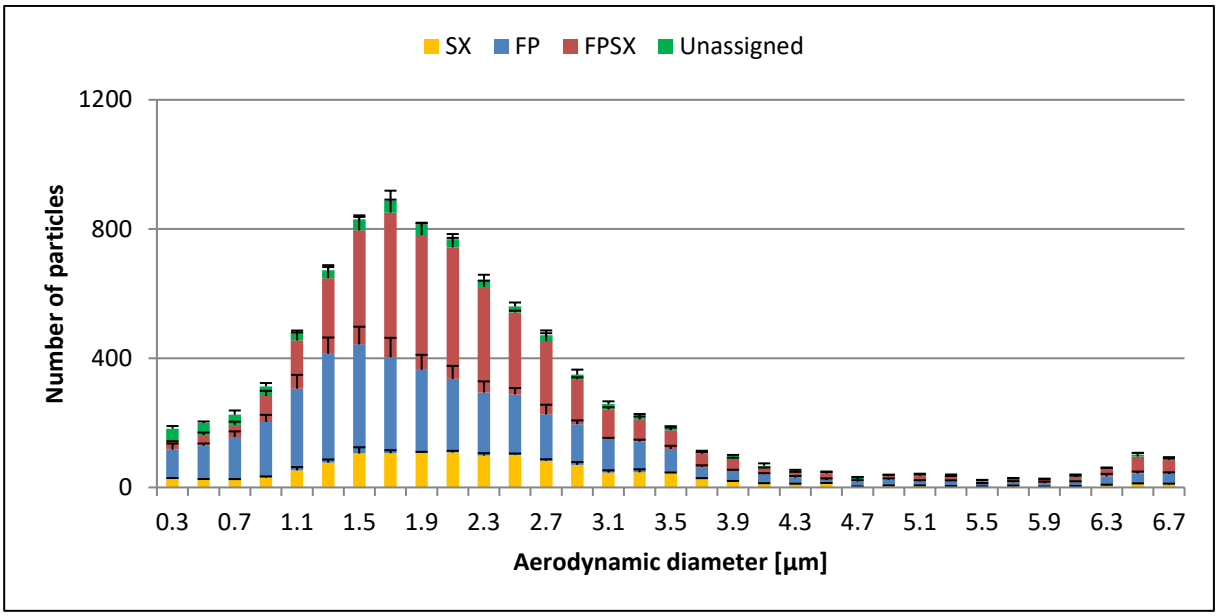

Figure 4 Aerodynamic particle size distribution of Advair Diskus® DPI obtained by SPAMS (n=3 DPI actuations; error bars represent one standard deviation). The plot shows the distribution of number of particle co-associations (FPSX), pure FP, pure SX and unassigned particles.

Table 1 Percentage of individual particles measured in the DPI formulations (10'240 particles per run).

[%]	Axotide Diskus® (FP)	Serevent Diskus® (SX)	Advair Diskus® (Combination)
FPSX	---	---	41.3
FP	79.1	---	38.8
SX	---	91.5	14.4
Unassigned	20.9	8.5	5.4

Axotide Diskus® and Serevent Diskus® had a median aerodynamic diameter (MAD) of 2.06 µm and 1.86 µm, respectively. The DPI combination product Advair Diskus® had an MAD for both APIs combined around 2.02 µm (Table 2). For the co-associated FPSX particles, the MAD is somewhat coarser than the free FP particles at approximately 2.02 µm, strongly indicating that those particles result from the agglomeration of fine SX and coarser FP particles. However, the MAD of pure FP was coarser in the DPI mono formulation than in the combination product. It may be possible that the lactose is changing the adhesive/cohesive behaviour of the APIs in the tested products [9].

Table 2 Median aerodynamic diameter (MAD) calculated from the DPI products measured by SPAMS.

MAD [µm]	Axotide Diskus® (FP)	Serevent Diskus® (SX)	Advair Diskus® (Combination)
FPSX	---	---	2.02
FP mono	2.06	---	1.36
SX mono	---	1.86	2.12
Overall	2.06	1.86	2.02

The SX particles that were not co-associated were coarser than FP particles, which were not co-associated. There may be a greater tendency of SX particles with small aerodynamic diameters to co-associate with coarser FP in the DPI combination product. In Axotide Diskus® the MAD for FP is 2.06 µm whereas the MAD for FP in Advair Diskus® is around 1.36 µm. This can be seen clearly in Figure 5 where it appears that primarily larger particles of FP are involved in co-association with SX particles. Also shown in Figure 5 is the comparison of Serevent Diskus® and the pure SX particles from Advair Diskus®. The MAD for Serevent Diskus® SX particles is 1.86 µm and for the pure SX particles in Advair Diskus® it is 2.12 µm, indicating the finer particles are the ones involved in co-association.

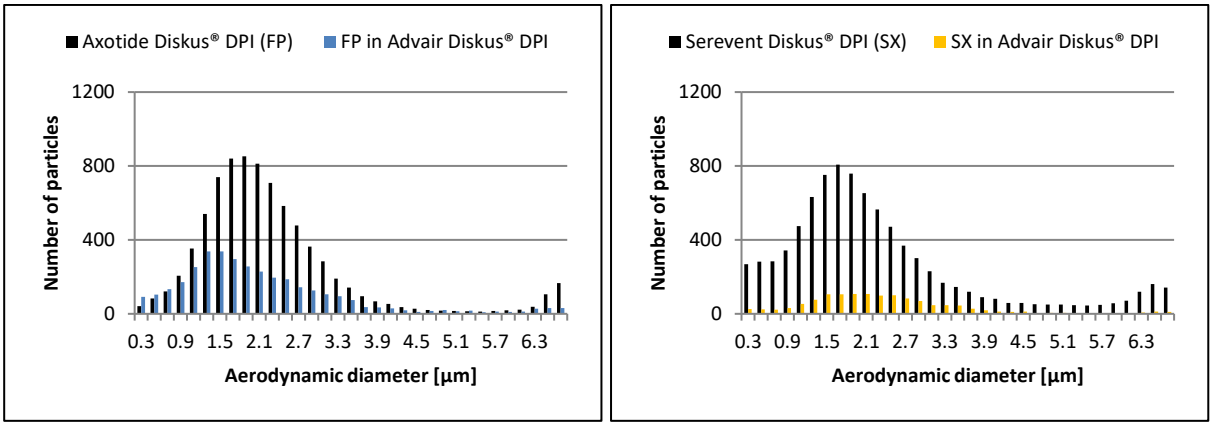

Figure 5 Overlay of aerodynamic particle size distribution of mono DPI products (left: Axotide Diskus® and right: Serevent Diskus®) with pure FP and SX found in Advair Diskus® DPI.

API co-associations in DPIs can arise from interactions between different API-containing particles as a result of high shear forces during the blending process or via the formation of weak agglomerates of API with lactose fines [10]. In the case of the Advair Diskus®, the APIs were processed together and could furthermore also mix in the aerosol plume following the actuation of the device. Soft agglomerates of API and fine lactose formed during device actuation that could effectively co-associate the two APIs without directly binding of the one API to the other. Nevertheless, this co-association could well deliver both APIs to the same site in the patient. Adding lactose to the blend as an excipient would presumably reduce the level of co-association by acting as a diluent. These and other mechanisms contributing to the levels of API co-association in inhaled products merit further study to optimize that co-association for increased pharmacological efficacy [3].

Conclusions

The collected data confirmed that high levels of co-associated FP-SX particles are emitted from Advair Diskus® examined after actuation of the device. Using the SPAMS technique revealed that approximately 41% of the respirable particles in the tested DPI product contained both FP and SX. Unique mass spectral fragmentation patterns were recognized and assigned for each API using software developed specifically for the analysis of SPAMS data. A large fraction of the detected SX was found in co-association with FP while a large fraction of pure FP particles was also detected. A significant finding was also that coarser FP particles and finer SX particles were preferentially forming the particle co-associations. There is high potential for the association of FP or SX or both with lactose or other excipients present in a formulation and future work should involve the development of techniques to further differentiate, identify and quantify both APIs and excipients at the level of the individual particle. Using SPAMS, we were able to reveal particle co-associations successfully in a combination DPI product and provides an interesting tool for formulation design.

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