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Research Paper

# Particle interactions of fluticasone propionate and salmeterol xinafoate detected with single particle aerosol mass spectrometry (SPAMS)

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ABSTRACT

Particle co-associations between the active pharmaceutical ingredients fluticasone propionate and salmeterol xinafoate were examined in dry powder inhaled (DPI) and metered dose inhaled (MDI) combination products. Single Particle Aerosol Mass Spectrometry was used to investigate the particle interactions in Advair Diskus® (500/50 mcg) and Seretide® (125/25 mcg). A simple rules tree was used to identify each compound, either alone 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 drug particle co-association (fluticasone-salmeterol) were observed in the aerosols emitted from Advair Diskus® and Seretide®. The majority of the detected salmeterol particles were found to be in co-association with fluticasone in both tested devices. Another significant finding was that rather coarse fluticasone particles (in DPI) and fine salmeterol particles (both MDI and DPI) were forming the particle co-associations.

## 1. Introduction

The two most common chronic respiratory diseases are asthma and chronic obstructive pulmonary disease (COPD). According to the 2016 estimate by the World Health Organization, 235 million people currently suffer from asthma and over 3 million people die each year from COPD, accounting for an estimated 6% of all deaths worldwide (WHO, 2017). Two major classes of drugs widely used in the treatment of these diseases are inhaled corticosteroids (ICS) and beta2-agonists (LABA), both preferably being delivered directly to the lungs by inhalation. The active pharmaceutical ingredients (APIs) are typically administered to patients via either a pressurized metered dose inhaler (pMDI) or a dry powder inhaler (DPI) device. The two classes of compounds have very different modes of action, targeting different aspects of the respiratory disease process (airway inflammation in the case of ICS and smooth muscle dysfunction in the case of LABA). Thus these two classes of drugs in combination address complementary aspects of the disease pathophysiology of asthma that neither drug class is able to achieve alone (Barnes, 2002).

Clinically, prescribing both classes of drugs has become a method of taking advantage of their complementarity. There is, however, 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 synergetic effect with improved clinical outcomes. Studies have shown increasing evidence of complementary and synergistic effects of LABA and ICS, interacting at the molecular receptor (Baraniuk et al., 1997; Mak et al., 1995; Michael et al., 2001; Usmani et al., 2002; Johnson, 2002) and cellular (Orsida et al., 2001; Dowling et al., 1999; Korn et al., 2001; Pang and Knox, 2000) level. Previous studies have ruled out systemic pharmacokinetic or pharmacodynamic interactions between inhaled fluticasone propionate (FP) and salmeterol xinafoate (SX) when administered in combination (Kirby et al., 2001). One postulated reason for this synergetic increase in pharmacological efficacy is the co-association of APIs at the point of deposition within the lung (Haghi et al., 2013; Taki et al., 2011). Specifically, salmeterol was observed to retard the transport of fluticasone across cell cultures of lung epithelial tissues, prolonging its localized anti-inflammatory effects (Haghi et al., 2013). The co-administration of the two drugs is expected to improve the likelihood of the two APIs being delivered to the same location in adequate concentrations, maximizing the likelihood of these synergetic effects (Nelson et al., 2003). Partly for patient convenience and partly to take advantage of this synergetic phenomenon, “combination products” have been introduced which contain both APIs in a single formulation administered simultaneously by a single device.

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Interestingly, the presence of one API in these products was demonstrated to affect the delivered dose of the other that reached the lung (Taki et al., 2011; Nelson et al., 2003; Traini et al., 2007; Gordon and Panos, 2010; Frampton, 2014) but that phenomenon was ruled out as the cause of the improved clinical outcomes for the combination products versus the administration of two single API products (Ashurst et al., 1998; Malley et al., 1998), indicating that the postulated synergetic phenomenon is, indeed, taking place in vivo. Previously, it was demonstrated that there is some affinity between fluticasone and salmeterol in solution, indicating that they may also be co-associated at the level of the individual administered aerosol particle (Michael et al., 2000). Attempts have been made to observe particle co-associations with Raman microscopy by (Theophilus et al. (2006). 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 (Morrical et al., 2015).

The current pharmacopoeial standard method for determining the aerodynamic particle size distribution (APSD) of drugs delivered from inhaled pharmaceutical products is to generate a size-segregated sample of the particles in a cascade impactor, typically the recently developed Next Generation Impactor (NGI) (Marple et al., 2004; Marple et al., 2003a; Marple et al., 2003b), followed by dissolving of each size-fractionated particle sample into a solvent which is then analyzed by high-performance liquid chromatography (HPLC). While highly quantitative for the total concentration of API delivered, this technique does not yield any information regarding the relationships between the various product components (API(s), excipients) within the formulation.

Mass spectrometry based aerosol analytical techniques have been undergoing continuous development since the 1970s (Prather et al., 1994; Su et al., 2004; Zhou et al., 2014; Zhou et al., 2016; Zhou et al., 2011). Aerosol-specific mass spectrometers such as the Livermore Instruments SPAMS 3.0 are capable of providing both aerodynamic particle size distribution (APSD) profiles of particles and the chemical composition of particle statistical sampling of 10,000 single particles in two minutes.

The SPAMS is descended from an earlier instrument, the Aerosol Time-of-Flight Mass Spectrometer, that was also capable of determining the aerodynamic size and chemical composition for individual particles in real-time, first reported by Prather et al. (1994). This instrument was initially developed for environmental and air pollution studies but was applied to the analysis of inhaled pharmaceutical products soon after its invention (Marple et al., 2003c; New et al., 2008).

Recently, SPAMS was developed as a more advanced instrument capable of characterizing single aerosol particles, providing both the aerodynamic diameter and chemical composition of each measured particle. The SPAMS has a very high rate of data acquisition (up to 250 particles per second) compared to earlier aerosol MS designs measuring only 1–4 particles per second. By analyzing a large number of particles and using application-specific chemometric software, the instrument is able to determine mass loadings for particular components (both APIs and excipients), which can be compared to results obtained by NGI. Previous experiments using both ATOFMS and SPAMS have demonstrated that particle co-associations between drug product components could be evaluated in a way that is inaccessible via impactor techniques (Morrical et al., 2015; New et al., 2008; Fergenson et al., 2014). A deeper understanding about product performance and interactions can be gained, providing valuable information for combination products. If a higher degree of co-associated particles would indeed be beneficial for patients, product developers could design formulations with a greater degree of co-associated API. This makes the SPAMS technique an attractive alternative in the field of pharmaceutical aerosol analysis (complementary to NGI/HPLC-analysis).

The objective of these experiments was to determine the degree of particle co-associations between fluticasone and salmeterol and the mass distributions of the particles containing the two different APIs across multiple size ranges in real-time. Axotide® and Serevent®, each a single API product containing fluticasone and salmeterol, respectively, were used to identify unique marker peaks from mass spectral signals of each API.

**Table 1**
Overview of DPI and pMDI product groups used in this study.

| Brand Name | Inhaler | API/Dosage Strength |
|---|---|---|
| Advair Diskus® | DPI | Fluticasone Propionate/Salmeterol Xinafoate 500/50 mcg |
| Axotide Diskus® | DPI | Fluticasone Propionate 500 mcg |
| Serevent Diskus® | DPI | Salmeterol Xinafoate 50 mcg |
| Seretide® | pMDI | Fluticasone Propionate/Salmeterol Xinafoate 125/25 mcg |
| Axotide® | pMDI | Fluticasone Propionate 125 mcg |
| Serevent® | pMDI | Salmeterol Xinafoate 25 mcg |

## 2. Materials and methods

In the current study, commercially available inhalation drug products containing fluticasone propionate (FP) and salmeterol xinafoate (SX) were investigated using a SPAMS 3.0. Table 1 lists the products used in this study. The experimental matrix consisted of both pMDI and DPI fluticasone mono, salmeterol mono and fluticasone-salmeterol combination formulations.

Chemical structures for fluticasone and salmeterol are given in Table 2.

### 2.1. Single particle aerosol mass spectrometry

The SPAMS instrument has been described elsewhere previously, but a brief description will be given here (Morrical et al., 2015; Steele et al., 2008). Fig. 1 is a schematic of the SPAMS instrument as configured for DPI measurements. An inhalation device is fitted to a USP throat (UIP, MSP Corp., Minnesota, USA) which is, in turn, mated to an aerosol residence chamber. The chamber is necessary to mediate between the higher flow rate of the devices under actuation and the SPAMS which draws 1.16 liters/min through a KF-16 port at the bottom of the chamber. The relaxation chamber is also a source of sampling bias due to the loss of particles from wall adhesion and from gravitational settling of particles in the size range of interest (Wang and Maxey, 2006). In these experiments, no attempt was made to scale the data according to shape or transmission efficiency of the SPAMS inlet interface. The devices are actuated via a vacuum pump (HCP5, Copley Scientific Ltd., UK)/critical flow controller (TPK-2000, Copley Scientific Ltd., UK) which are connected through a third port in the side of the chamber.

The SPAMS system is maintained under vacuum. Aerosol particles are introduced from the chamber into its top through an inlet nozzle and proceed through a series of aerodynamic focusing lenses. The lenses collimate the particles into a beam and also serve to accelerate the particles in the beam to a terminal velocity that is a function of their aerodynamic diameters. The SPAMS determines each particle's size in real-time by measuring its transit time as it traverses a square profiled laser beam and its scattered light is detected by a photomultiplier tube (2). This transit time is compared to a calibration made from particles of known size and density, returning their aerodynamic diameters at the level of the individual particle. The light scattering event is also used to trigger the firing of a pulsed ionization laser which desorbs and ionizes the particle. The desorption/ionization process occurs at the center of the source region of a dual polarity time-of-flight mass spectrometer. Both positive and negative ion spectra are simultaneously acquired and stored along with that particle's transit time. Up to 250 of these mass spectra/aerodynamic diameters can be acquired per second, each from