White Paper

Using Raman Chemical Imaging to Analyze Inhaled Combination Therapies

Since the approval of the Seretide (Advair) fluticasone/salmeterol dry powder inhaler in 1999, inhaled therapies that combine a bronchodilator, often a long-acting beta agonist (LABA), with a maintenance drug, usually an inhaled corticosteroid (ICS), have taken over a large portion of the asthma and COPD markets. And, increasingly, a long-acting muscarinic antagonist (LAMA) is being added to LABA/ICS formulations to create triple therapy combinations.

Developers of dry powder formulations that contain multiple active ingredients are increasingly turning to scientists with expertise in Raman chemical imaging who, using a novel proprietary analytical method, are providing important data about the aerosolization behavior of these powder mixtures that was previously unavailable. This analysis offers developers new opportunities to optimize both the formulations and the delivery devices for combination therapies, potentially shortening development time and decreasing cost significantly.

Since the method is validated and performed in a cGMP laboratory by recognized experts in the field, it also can produce reliable data for registration studies that documents the stability of the formulation.

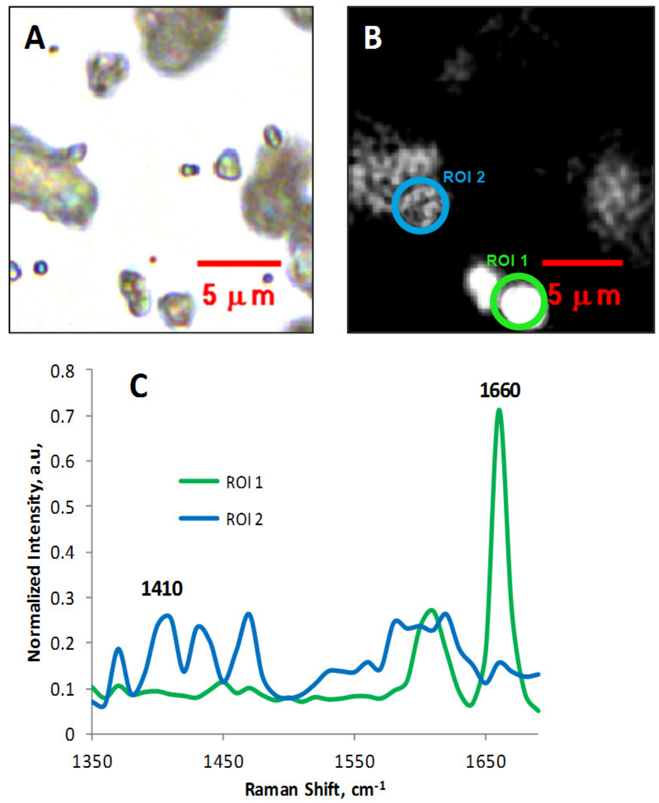

Figure 1. (A) Brightfield reflectance image and (B) processed RCI image of particles and (C) their respective Raman spectra.

The Challenges of Analyzing Combination Formulations

For the therapies to work effectively, the ratio of drugs and the PSD of each active ingredient in the respirable fraction must meet specification and must be consistent from actuation to actuation. Because multiple-API inhalation products often also contain a carrier such as lactose, the aerosolization behavior of the formulation usually depends on interactions between three or four different powders, each with different particle size distributions and surface characteristics. The complexity of these interactions makes it difficult to predict how the formulations will behave under various conditions.

The 1998 FDA draft guidance that deals with dry powder inhalers (DPIs) states, “Regardless of the DPI design, the most crucial attributes are the reproducibility of the dose and particle size distribution” and recognizes that maintaining that reproducibility over the lifetime of the product is a “formidable challenge.”

Without a thorough understanding how each component of a fixed dose combination DPI behaves in relation to the other during aerosolization and after deposition, meeting the reproducibility and stability challenge requires a costly, time-consuming trial and error process. Even simply demonstrating reproducibility for regulatory submissions can be difficult without data on the PSDs of the individual APIs, which may be impossible to determine accurately using standard methods such as cascade impaction and optical microscopy.

PSD data produced by cascade impaction cannot differentiate between ingredients or distinguish large particles from aggregates, and difficulties in controlling moisture, air flow, and actuation can affect the reproducibility of cascade impaction results. Optical microscopy is subjective, and differences in particle composition can be very difficult to spot. In addition, the dosage of one API may be significantly larger than the other, meaning that only a very small amount of the second API may be present in any given sample subjected to particle sizing analysis.

Because the interactions of the various components depend largely on the surface energies of the particles, some researchers have attempted to gain a better understanding of aerosolization behavior by using the colloid probe method of atomic force microscopy (AFM) to determine the cohesive-adhesive balance (CAB) between each of the components of a dry powder formulation.

In theory, knowing the CAB between each drug and the lactose and the CAB between each of the drugs should allow analysts to predict how much of each drug will detach from the carrier, and therefore the ratio of the individual components in the fine particle fraction. In practice, however, variations in factors such as morphology and moisture content can significantly affect the surface energies of the particles, making predictions of aerosolization behavior unreliable.

The Raman Chemical Imaging Method

Now, experts in Raman chemical imaging (RCI) have developed a method that allows them to reliably and reproducibly measure the PSDs of each of the active ingredients found in the fine particle fraction after actuation of the device, the amount of each drug that remains attached to carrier particles, and the number of drug particle aggregates.

RCI takes advantage of the fact that each type of drug molecule emits a characteristic signature and therefore can be identified through analysis of its spectrum. The RCI system combines spectral information gathered by Raman spectroscopy with morphological information obtained by optical imaging, allowing highly trained analysts to assess the composition of the powder mixture found in the fine particle fraction.

With the ability to detect spectral shifts as small as 2 cm^{-1}, the RCI method is even sensitive enough to allow scientists to identify individual polymorphs of drugs. This ability is critical because different polymorphs have different dissolution rates, and the presence of particular polymorphs in the formulation therefore affects bioavailability.

Method development begins with the creation of a Raman spectral library. Analysts must take factors such as fluorescence and Raman scattering signal strength into account in order to determine testing parameters, and their experience in selecting the correct factors ensures that the method will accurately identify each active ingredient in the formulation and, if desired, the carrier.

After identification of the spectra of the relevant components, a technician collects samples for ingredient-specific particle sizing (ISPS), actuating a dry powder inhaler containing the combination formulation into the inlet of a Next Generation Impactor (NGI) and then sampling the powder deposited on a selected stage of the impactor, approximately in the mid-range of the respirable fraction. The powder collected is then dispersed onto an aluminum slide.

The RCI microscope collects data from hundreds of randomly selected fields of view from across each sample. The system then overlays the spectral data with an optical image of the sample, produces data correlated on a pixel-by-pixel basis that clearly shows which particles consist of excipients or any of the active ingredients **(Figure 1)**, and calculates particle counts and sizes for each component.

The operator then reviews and interprets composite images generated by the system to identify stand-alone or aggregated particles, either drug/drug or drug/carrier aggregates. After identification by the operator, particles of interest are automatically counted and sized.

As formulations with increasing numbers of active ingredients are created, with or without carriers or other excipients, the ability of recognized experts in Raman chemical imaging to analyze and document the aerosolization behavior of the individual components will continue to simplify the development process.

This work was performed in collaboration with Chris Vernall, Robert Price and Jagdeep Shur from the Pharmaceutical Surface Science Research Group, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK.