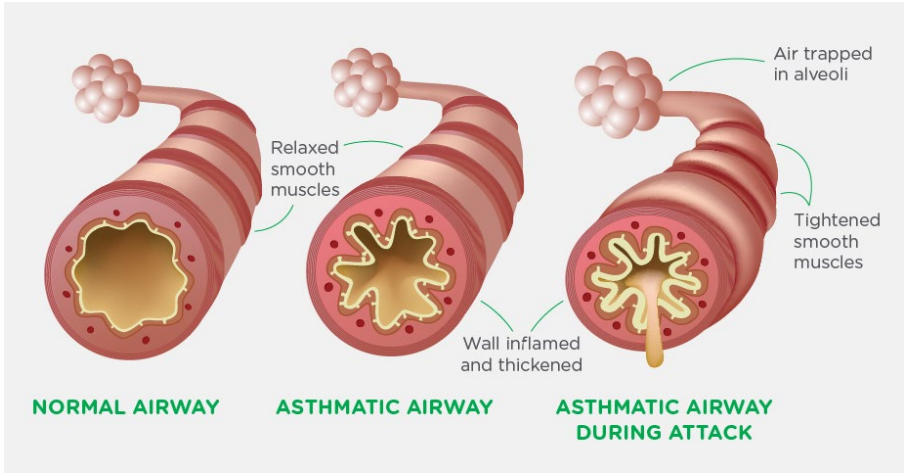
UAB SCHOOL OF ENGINEERING
The University of Alabama at Birmingham



UAB HEERSINK SCHOOL OF MEDICINE
The University of Alabama at Birmingham

Materials for Inhaled Aerosol Treatment of Disease: Can O-PTIR Microscopy be employed as a Tool to Evaluate the Complex Powder Nanostructure?

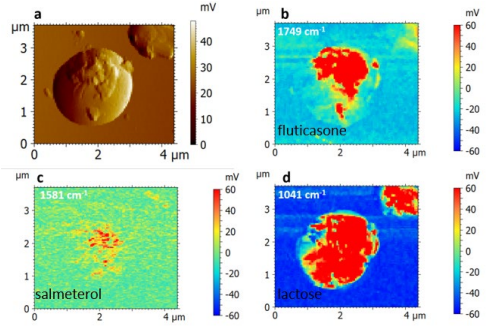
Mark Banaszak Holl

Department of Mechanical and Materials – School of Engineering
Division of Pulmonology, Allergy, and Critical Care Medicine – Heersink School of Medicine

SciX October 2025

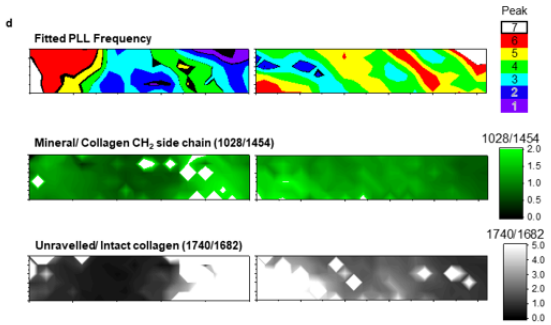
Banaszak Holl Lab Photothermal Infrared Applications

Drug Delivery



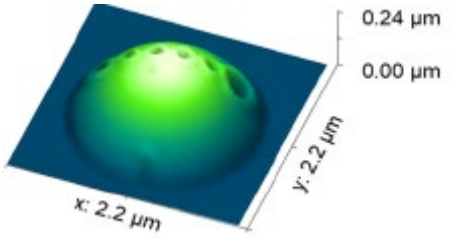
Analytical Chemistry **2025**, *97,* 16421.
International Journal of Pharmaceutics **2024**, 656, 124116.
International Journal of Pharmaceutics **2023**, *646,* 123505
Advanced Drug Delivery Reviews **2023** *192*, 114646
International Journal of Pharmaceutics **2023** *632*, 122563
Analytical Chemistry **2020** *92*, 8328-8322

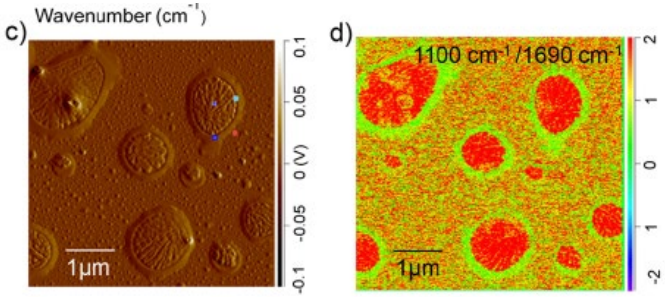
Bone and Ligament



Communications Biology **2023**, 6, 564
Journal of Structural Biology **2022**, 3, 107878
American Journal of Sports Medicine **2019**, 47, 2067

Microplastics, Materials & Environmental Aerosols





Cellulose **2024**, 31, 765
Int. J. Biol. Macromol. **2024**, *254*, 127972
ACS Sustain Chem Eng **2023** *11,* 12541
J. Env. Management, **2023** *343*, 118205
Analytical Chemistry **2022** 94, 11973
Macromolecules **2022** 55, 5301
ACS Earth and Space Chemistry **2022** 6, 871

Chemical Engineering Journal **2022** 428, 132614
ACS Applied Materials & Interfaces **2021** *13*, 46033
ACS Nano **2021** *15*, 13721
ChemPhotoChem **2021** 5, 571
Polymer **2021** *222*, 123643
Frontiers in Marine Science **2020** *7*, 576170
Global Challenges **2019** 1800104
Analytical Chemistry **2017** *89*, 8594

Bruker IconIR
AFM-IR

Photothermal mIRage-LS
O-PTIR

Engineering for the Human Process - Aerosols for Drug Delivery

University of Alabama at Birmingham

School of Engineering

Department of Mechanical and Materials Engineering

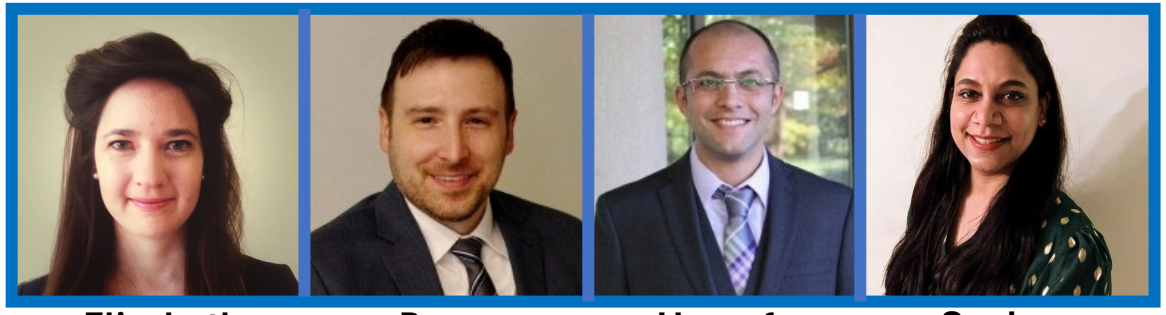
Division of Pulmonology, Allergy, and Critical Care Medicine

Lung Health Center

Heersink School of Medicine

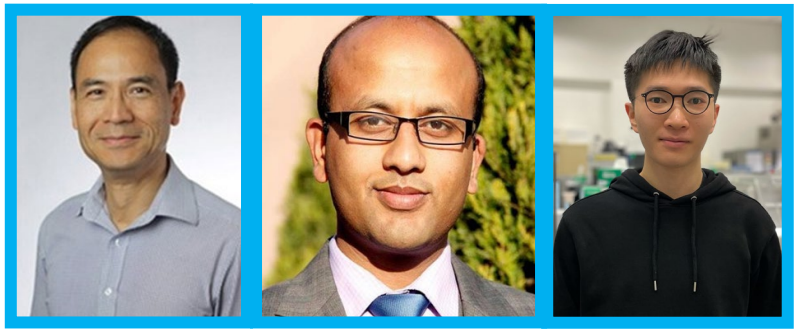
Mark Banaszak Holl | Sheikh Tanzina Haque | Blessy Joseph | Terri Ross | Thanh Kien Ta

U.S. Food and Drug Administration



Elizabeth Bielski | Bryan Newman | Huzeyfe Yilmaz | Snober Ahmed

University of Sydney School of Pharmacy



Hak-Kim Chan | Dipesh Khanal | Eric Cao

$$$ U.S. FDA Contract 75F40122C00202 Identification of Drug Distribution in Aerosols A Nanospectroscopy and NanoThermal Analysis

Asthma

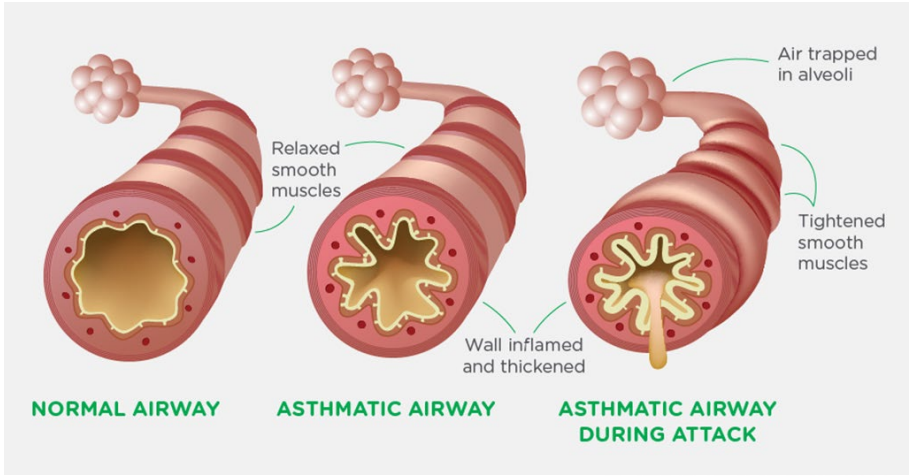
Chronic, inflammatory disease of conducting airways affecting 300 million people worldwide. Affluent societies have occurrence in 1 in 10 children and 1 in 12 adults. Estimated economic impact is estimated to exceed $18B. A mixture of genetic susceptibility and environmental risk factors lead to a variety of endotypes and consideration of asthma as a syndrome.

Both innate and adaptive immune systems act with epithelial cells to cause:

- Bronchial hyper-reactivity (non-specific response to exercise and cold air)
- Mucus overproduction
- Airway wall remodeling
- Airway narrowing

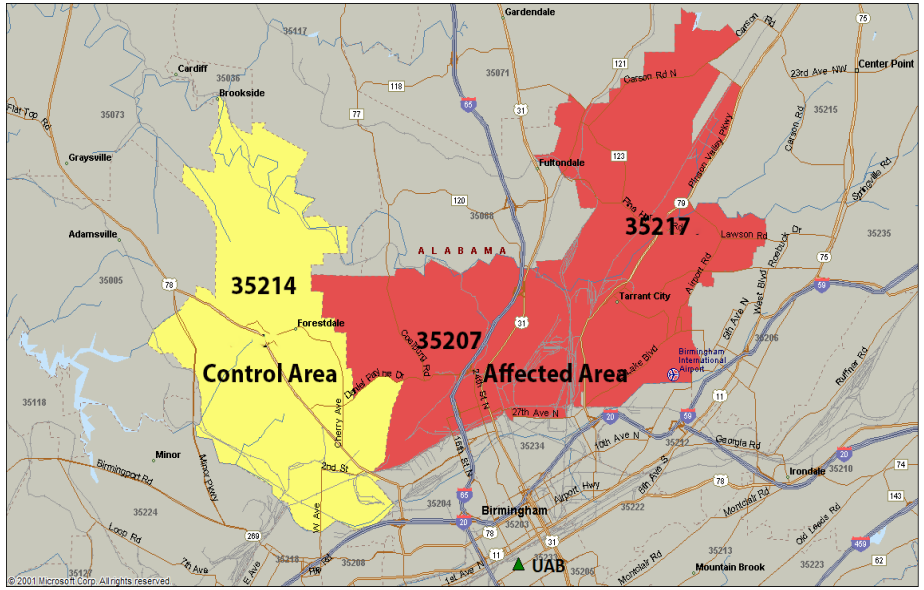
Leading to:

- Shortness of breath
- Wheezing
- Chest tightness



Lambrecht, Hammad *Nature* **2015**, *16*, 45-56.

Asthma & COPD: A Legacy of the Past in Birmingham, Alabama



LUNG DISEASE IS HIGHLY PREVALENT

The Affected Area (EPA superfund site) has twice the prevalence of COPD than Control Area.

74% Children in K-8th grade have physician diagnosed Asthma (7x national average!).

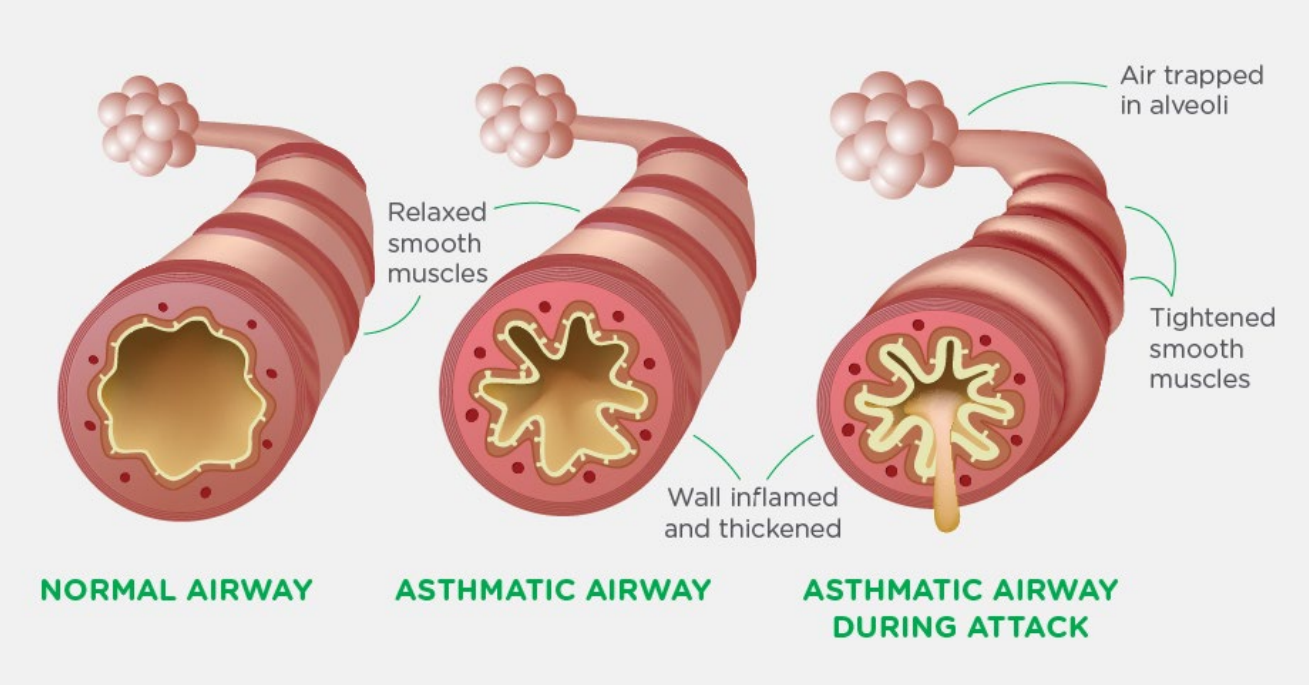
Current and Ongoing Health Crisis!

Major plants currently operating *in the city* including ABC Coke, ACIPCO, NUCOR.

ACIPCO in process of switching from coke to electric induction furnaces.

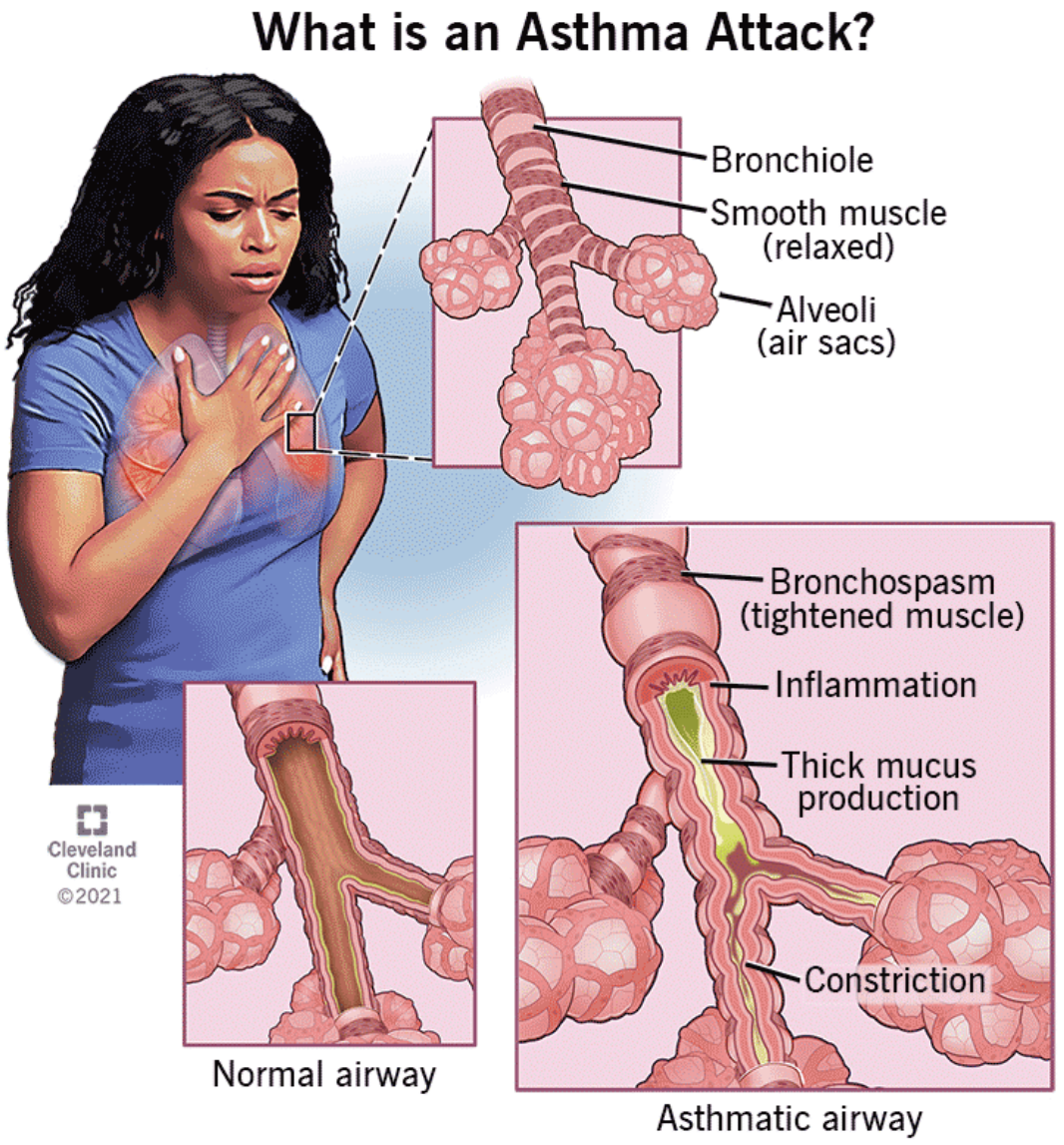
COPD: Chronic Obstructive Pulmonary Disease
EPA: U. S. Environmental Protection Agency

Asthma flare-ups are caused by triggers, which are irritants like high smog levels, colds and flu, cigarette smoke, scented cleaning products or even seasonal changes in the weather.



What happens during an asthma attack?

The airways get swollen and inflamed.
Thick, sticky mucus fills up the airways.
The muscles that wrap around the airways squeeze tight.
Air becomes trapped in alveoli.



https://www.choa.org/parent-resources/asthma/asthma-attacks

https://my.clevelandclinic.org/health/diseases/6424-asthma

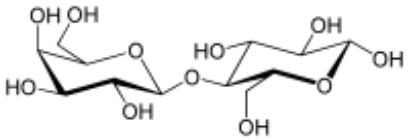
Inhaled Corticosteroid (ICS) and β2-agonist for Asthma Treatment

Corticosteroids (i.e., steroids): anti-inflammatory medicine. Synthetic version of hormones normally produced by the adrenal glands (two small glands that sit on top of the kidneys).

Effective for ~90% of Asthma cases

Fluticasone Propionate (FP): Flovent HFA, Flonase, and Flixotide

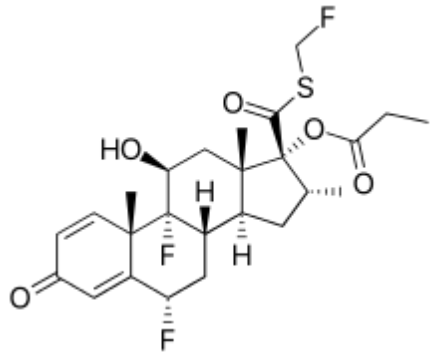
Mixed with lactose excipient



Fluticasone Propionate Metered Inhalation Aerosol (U.S. Marketed)

Fluticasone Propionate Metered Nasal Spray (U.S. Marketed)

Fluticasone Propionate Metered Inhalation Aerosol (EU, AUS, NZ marketed)

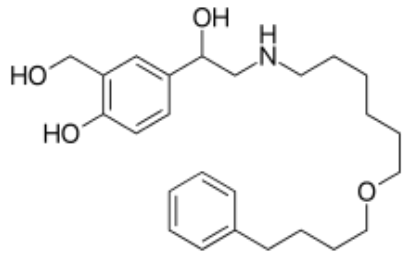


agonist at the glucocorticoid receptor producing anti-inflammatory and vasoconstriction effects.

β2-agonist:

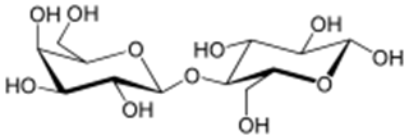
Salmeterol Xinafoate (SX): Serevent

Salmeterol Xinafoate Inhalation Powder (U.S. Marketed)

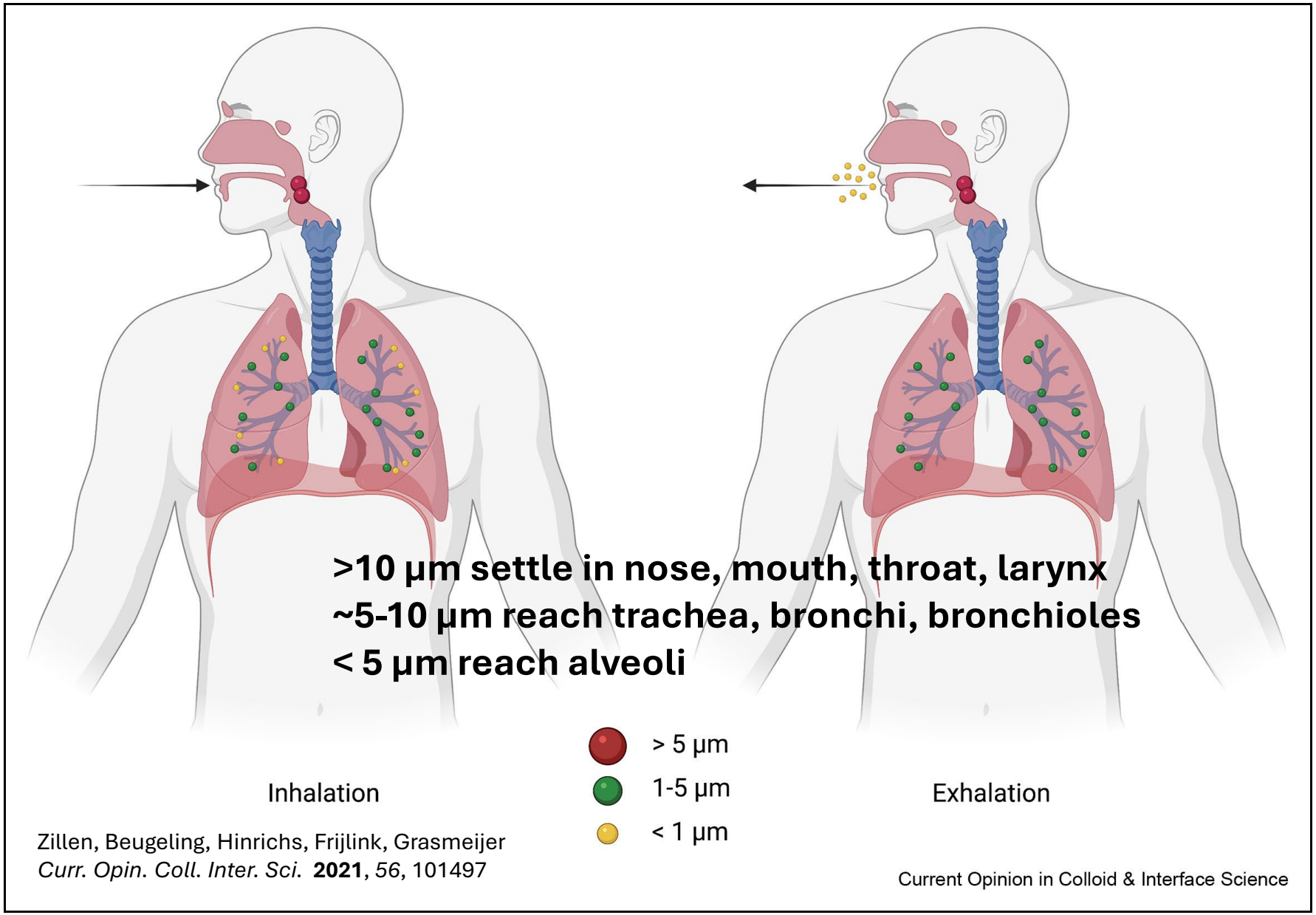


β2-agonist that relaxes bronchial muscles in the lungs – 12-hour action

Jetzer, M. W.; Morrical, B. D.; Fergenson, D. P.; Imanidis, G. *Int. J. Pharm.* **2017**, *532*, 218–228.



Dispersion into lungs with inhaler achieved by absorbing drugs on micron-size lactose particles.



Zillen, Beugeling, Hinrichs, Frijlink, Grasmeijer
Curr. Opin. Coll. Inter. Sci. **2021**, *56*, 101497

Current Opinion in Colloid & Interface Science

Advair Diskus (fluticasone propionate; salmeterol xinafoate) Inhalation Powder

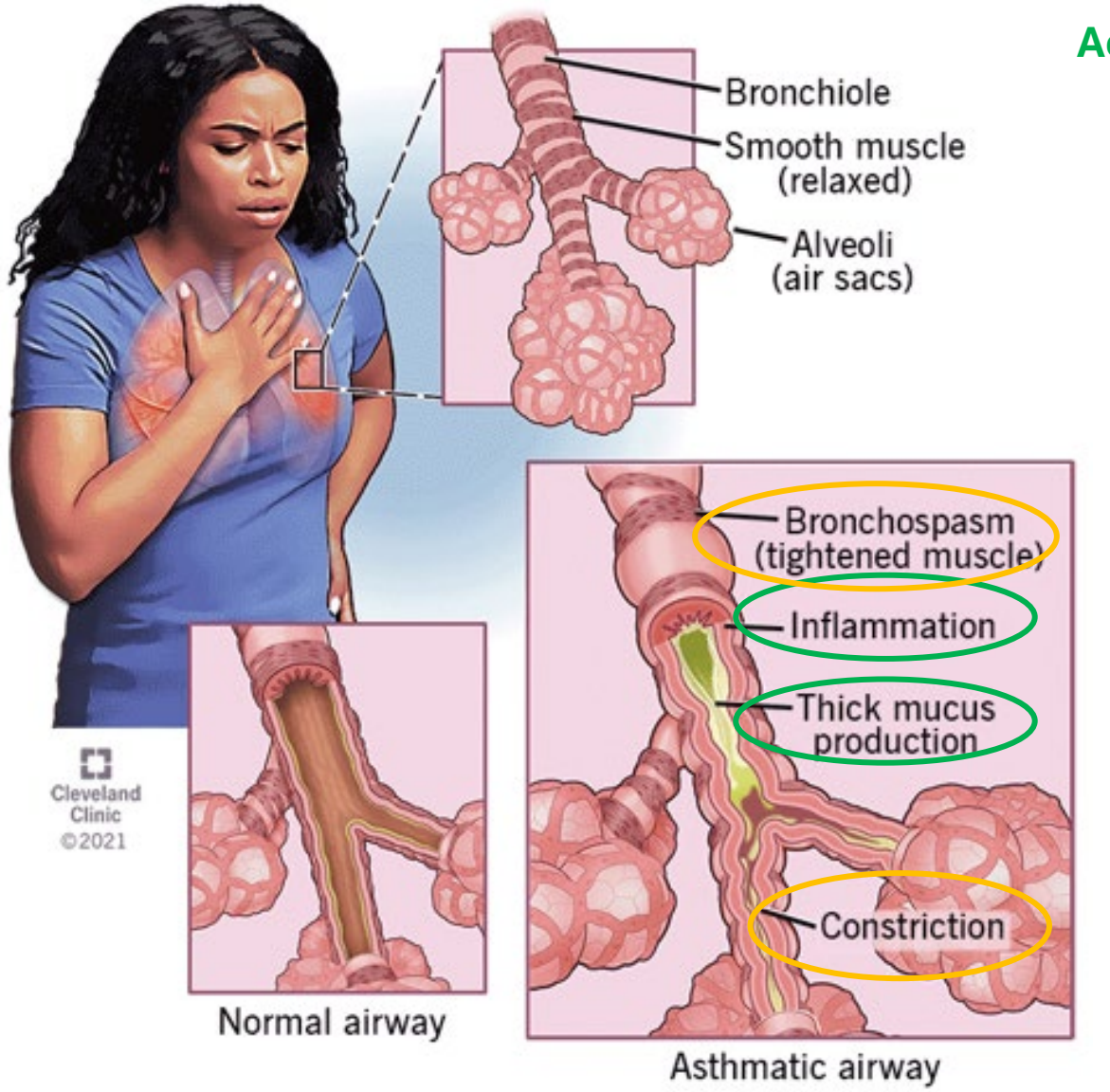
Deliver directly to lung cells to obtain therapeutic dose

while

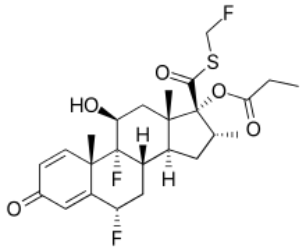
Reducing systemic delivery and associated side effects

Common: Headaches, sore throat, cramps, increased heart rate

Rare: allergic reaction, bone density loss, glaucoma, slowed growth (children)



Action of corticosteroid fluticasone

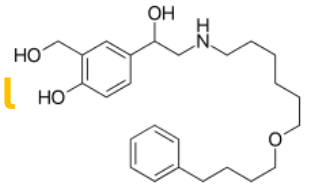


"significant clinical and in-vitro evidence to suggest that the co-association of **steroid** AND **β2-agonist** at the level of the single administered aerosol particle leads to a synergetic effect with improved clinical outcomes"

Synergistic effects operating at both the molecular receptor and cellular levels

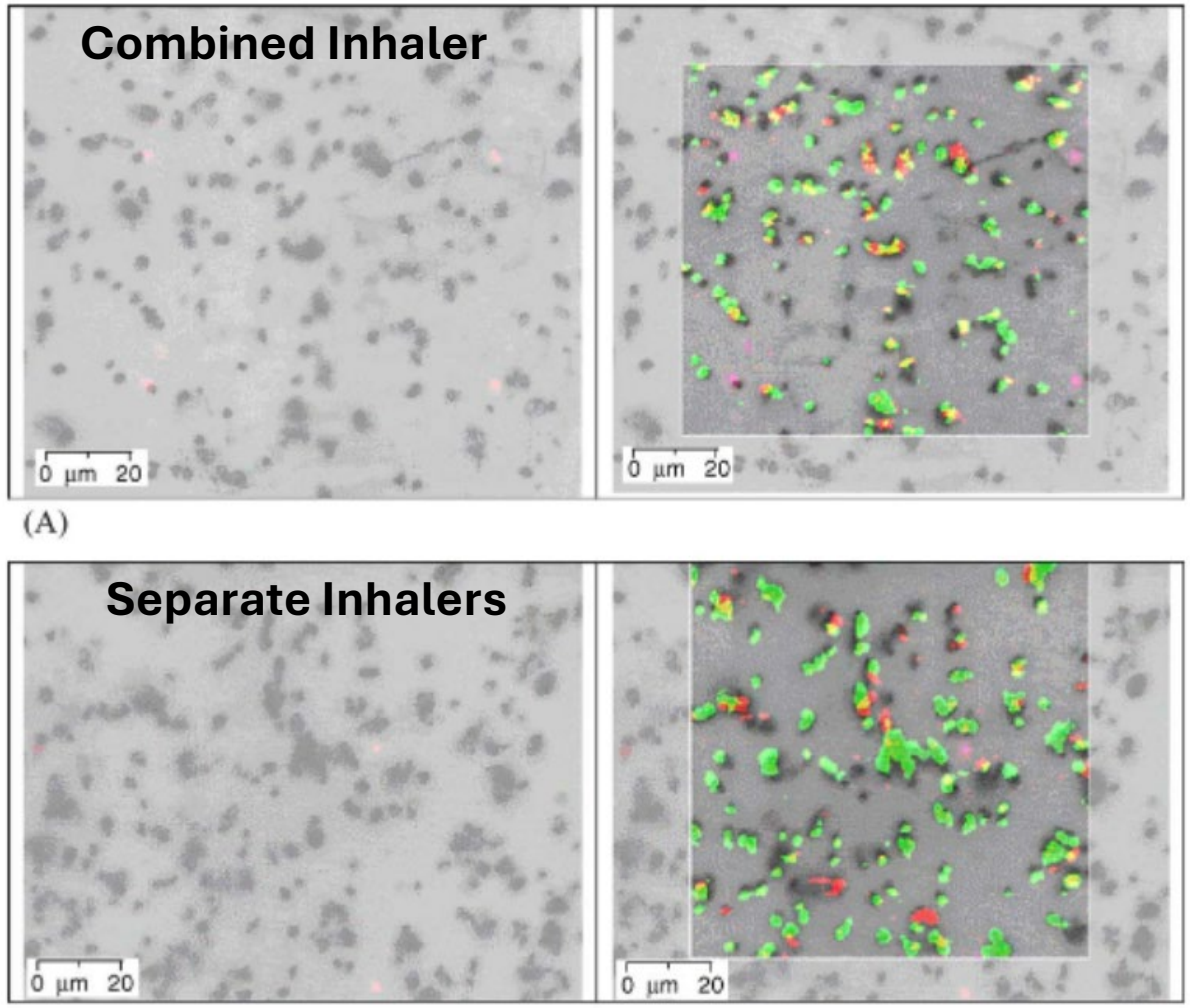
Important to deliver **BOTH** drugs to the **SAME** cell in the lung tissue

Action of β-2 agonist salmeterol



Jetzer, M. W.; Morrical, B. D.; Fergenson, D. P.; Imanidis, G. *Int. J. Pharm.* **2017**, *532*, 218–228.

What is known about Aerosol Particle Content and Structure ?



Fluticasone and Salmeterol Particle Co-Association by Raman Microscopy

Fluticasone Propionate
Salmeterol Xinafoate

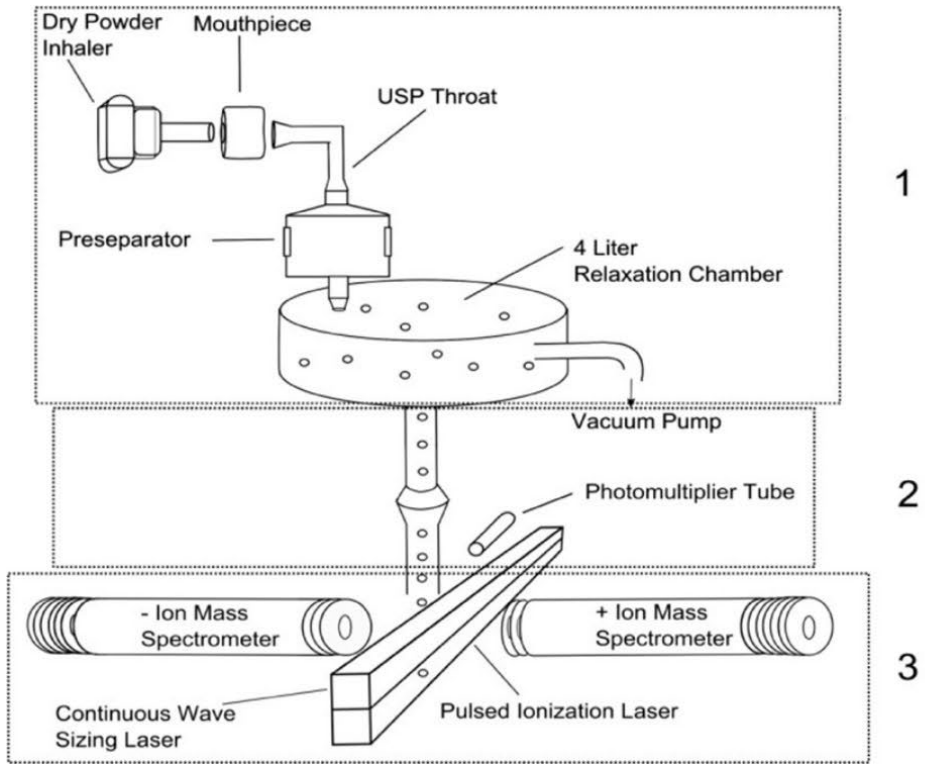
Drugs combined in particles Yellow

Spatial resolution ~ 3 µm or about size of many single particles

Theophilus, A.; Moore, A.; Prime, D.; Rossomanno, S.; Whitcher, B.; Chrystyn, H. *Int. J. Pharm.* **2006**, *313*, 14–22.

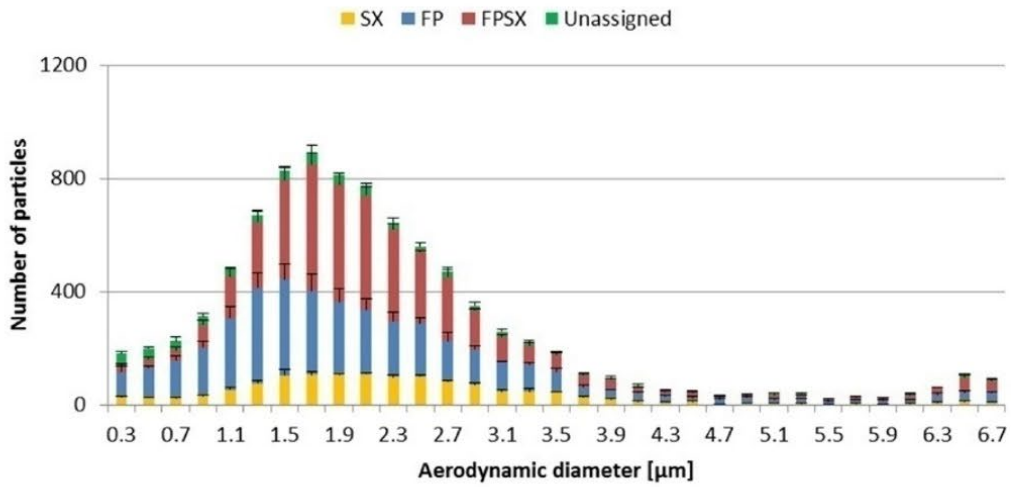
Fluticasone and Salmeterol Particle Co-Association by Mass Spectrometry

Novartis/University of Basel



Fluticasone Propionate : Salmeterol Xinafoate

10-Fluticasone/ 1-Salmeterol co-associated particles

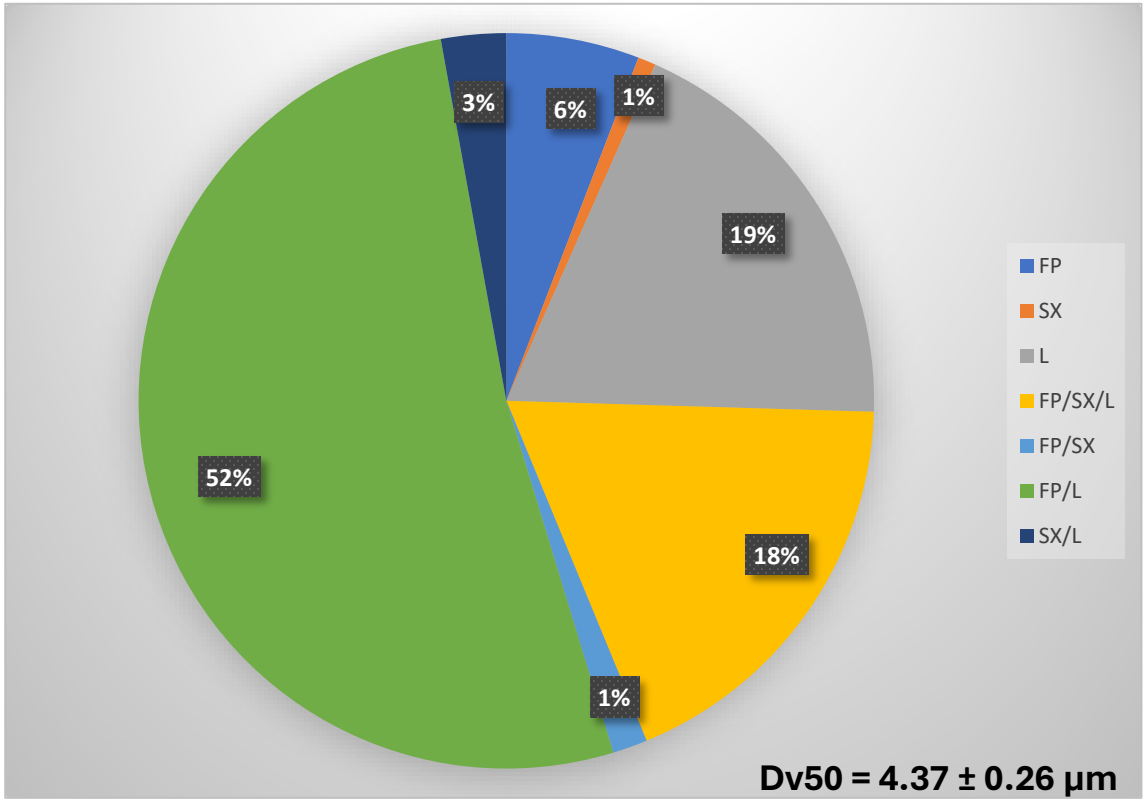


- **45% of particles contain fluticasone and salmeterol**
- **70% of particles containing salmeterol also contain fluticasone**

Jetzer, M. W.; Morrical, B. D.; Fergenson, D. P.; Imanidis, G. *Int. J. Pharm.* **2017**, *532*, 218–228.

Characterization of Advair Diskus (fluticasone propionate; salmeterol xinafoate inhalation powder) using Morphologically-Directed Raman Spectroscopy (MDRS)

Single Actuation, Next Generation Impactor (NGI) Stage 2



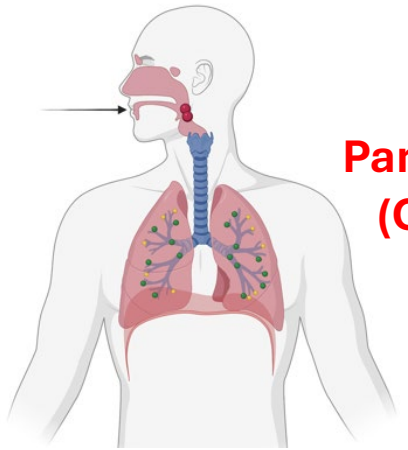
Chemical classification of agglomerates of Advair Diskus 100/50 (fluticasone propionate; salmeterol xinafoate inhalation powder, 0.1 mg/inh; EQ 0.05 mg base/inh) collected at 60 L min^{-1} with a USP inlet and pre-separator connected to the ADC apparatus. Quantities are a percentage by number and an average of 6 independent measurements of at least 3000 particles.

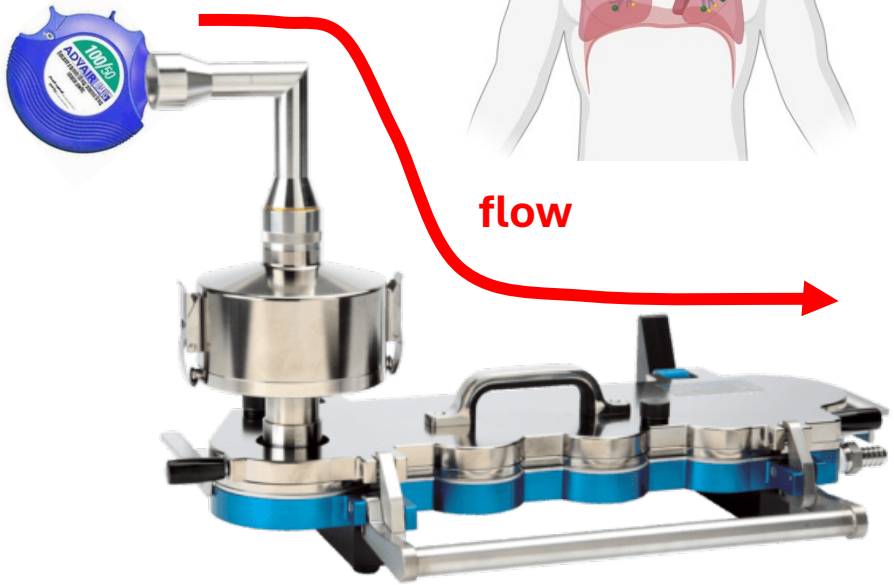
Dv50 is the mean diameter by volume

Farias G, Ganley WJ, Price R, Conti DS, Mangal S, Bielski E, Newman B, Shur J., *Pharmaceutical Research*. **2024** Oct;41(10):2015-29.

Evaluating Aerodynamics - Next Generation Impactor

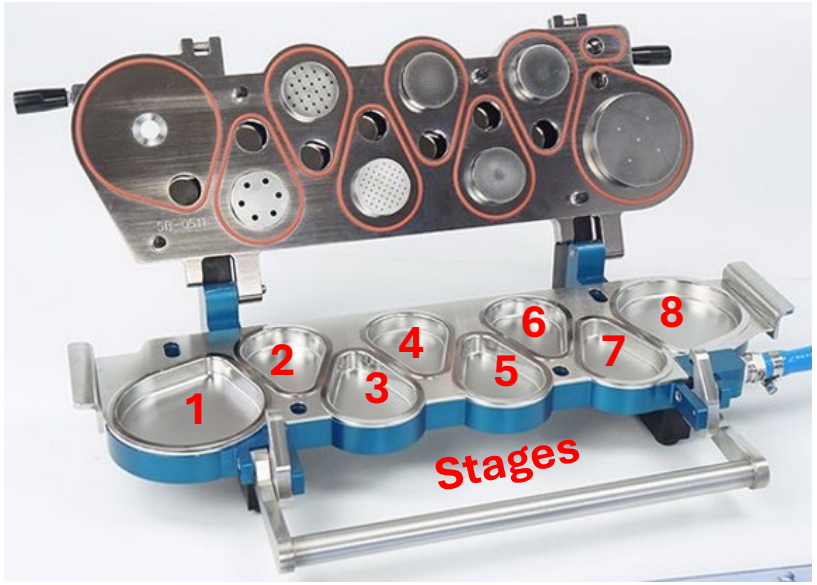
Particles In Here





flow

Particles collected on silicon (Si) wafers or calcium fluoride (CaF_2) cover slips at different stages as a function of size



Analysis

O-PTIR: mIRage-LS

AFM-IR: IconIR

Stage	1	2	3	4	5	6	7	8
Cutoff Diameter in microns	6.1	3.4	2.2	1.3	0.72	0.4	0.24	< 0.24

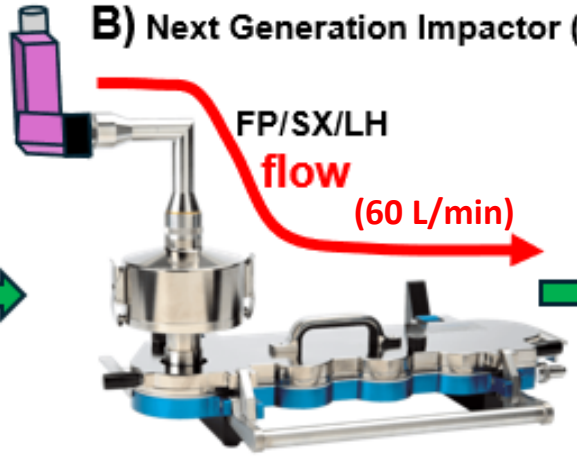
O-PTIR with Quantitative Morphology Analysis

A) Formulation Methods

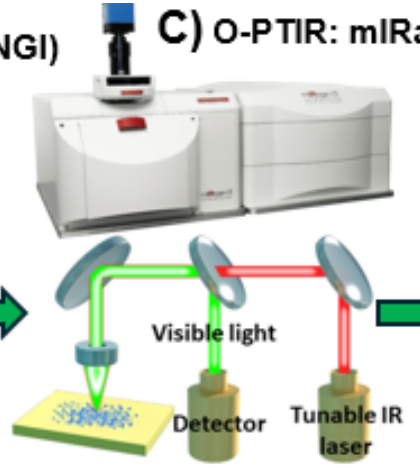
Turbula Mixer

Buchi S-300 Spray dryer

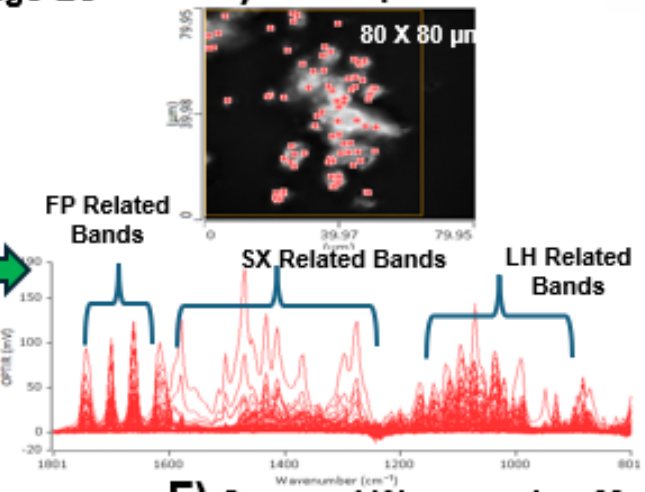
B) Next Generation Impactor (NGI)



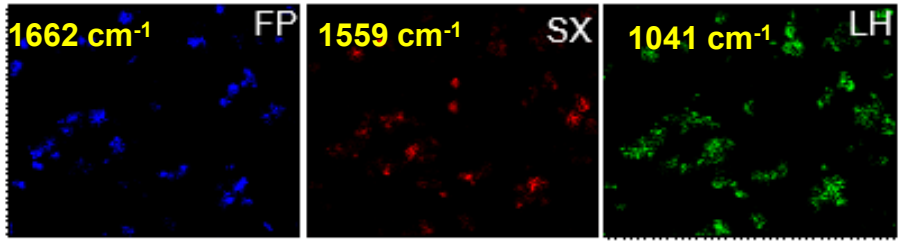
C) O-PTIR: mIRage-LS



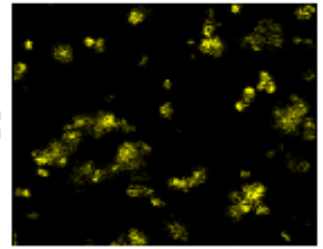
D) Point Spectra



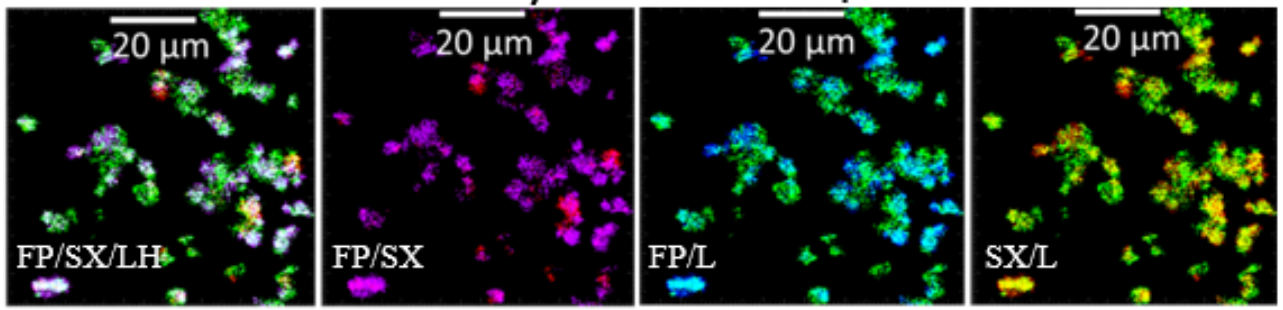
E) Single Wavenumber Maps



F) Summed Wavenumber Map

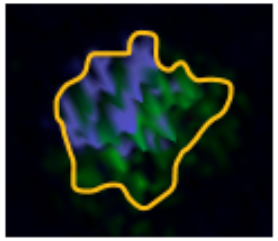


G) Colocalization Maps



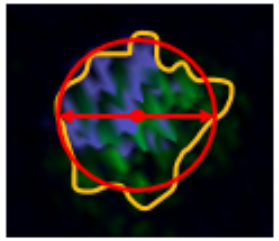
H) Key Morphological Parameters

Area



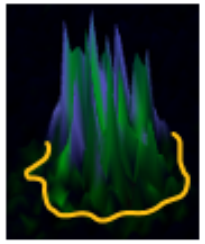
O-PTIR Single Wavenumber Defined Region

Equivalent Area Diameter



Diameter defined by particle area

Integrated Intensity Volume

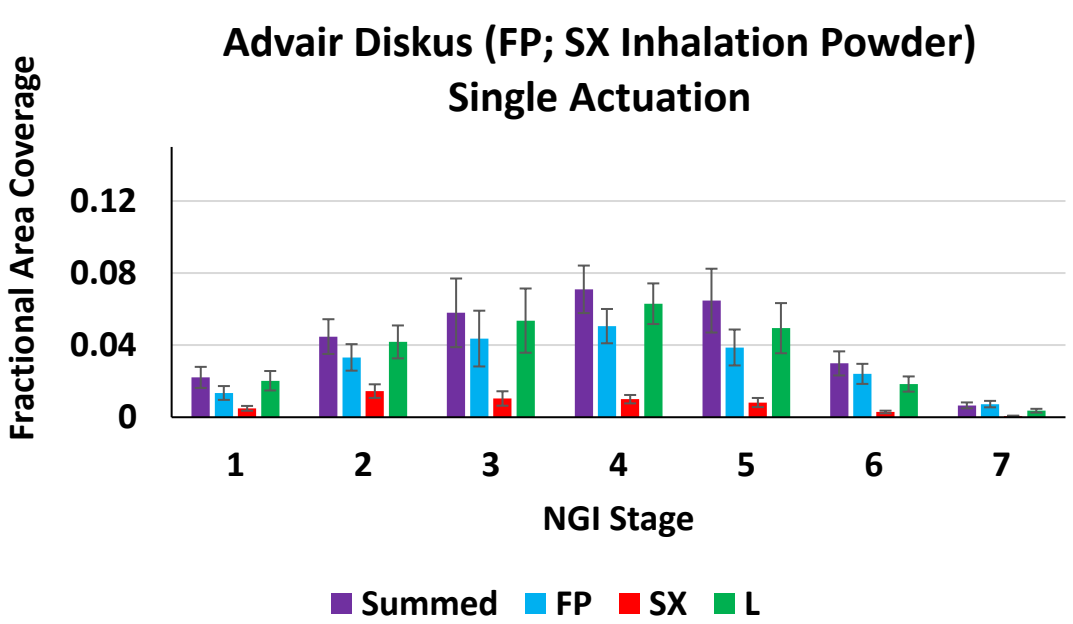


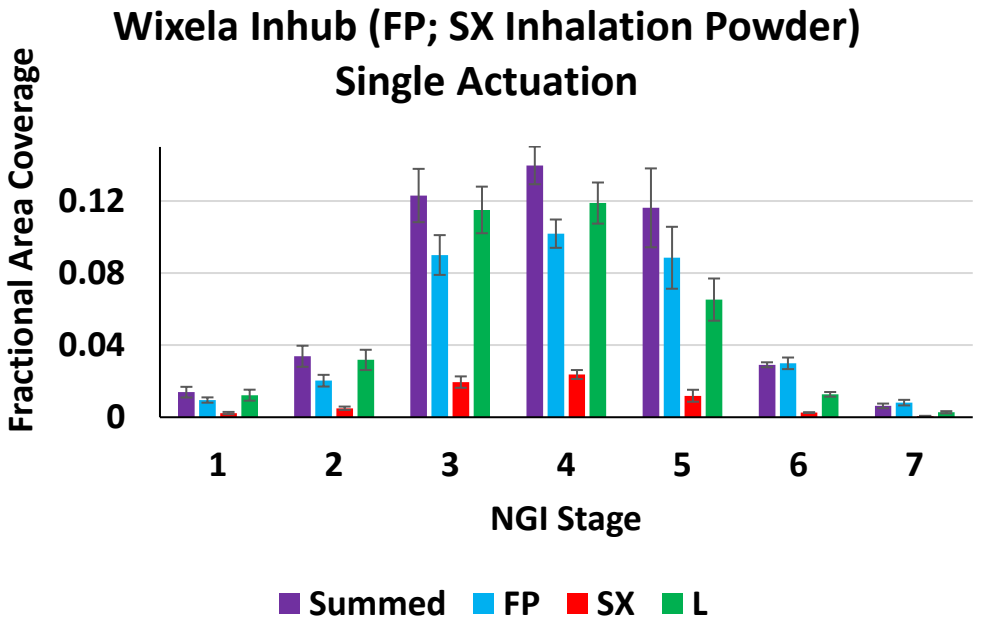
Volume sum of intensities at all particle pixels

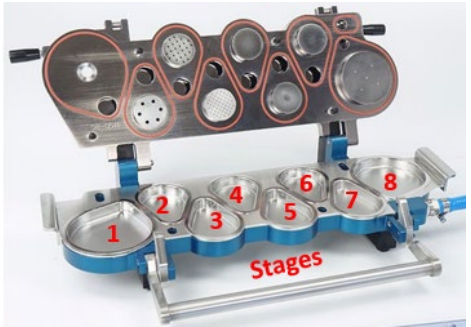
FP: fluticasone propionate; SX: salmeterol xinafoate; LH or L: lactose monohydrate

Fractional Area Coverage of Particles on CaF_2 Surface

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)





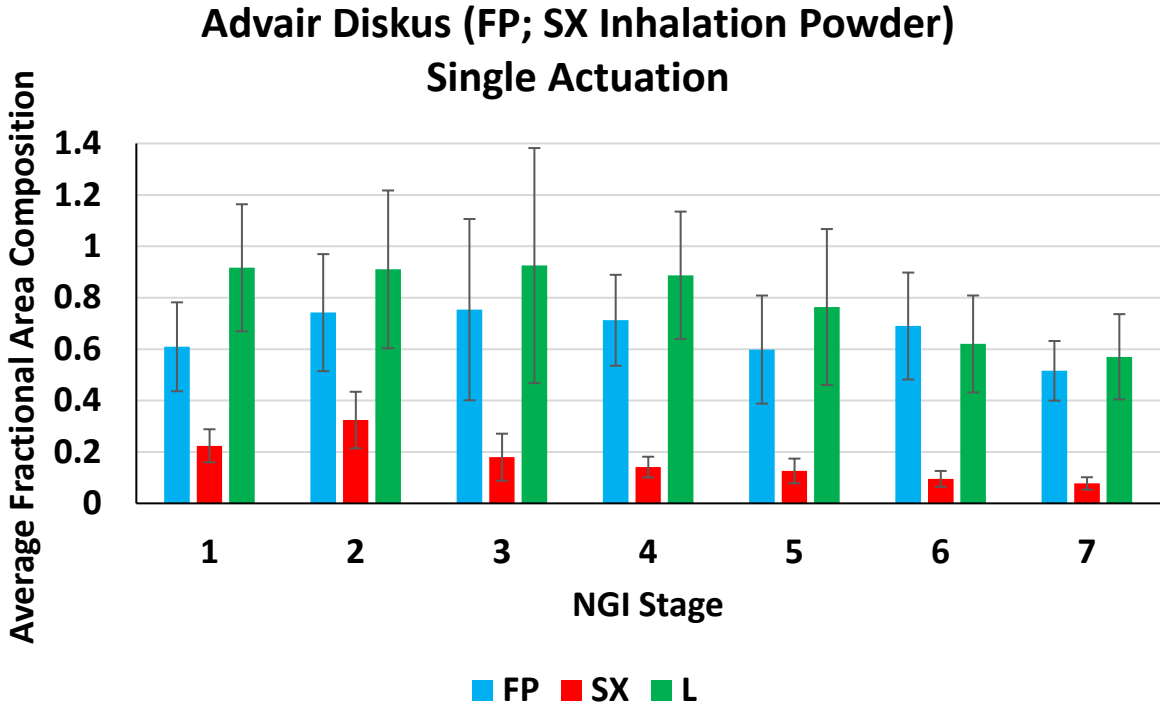


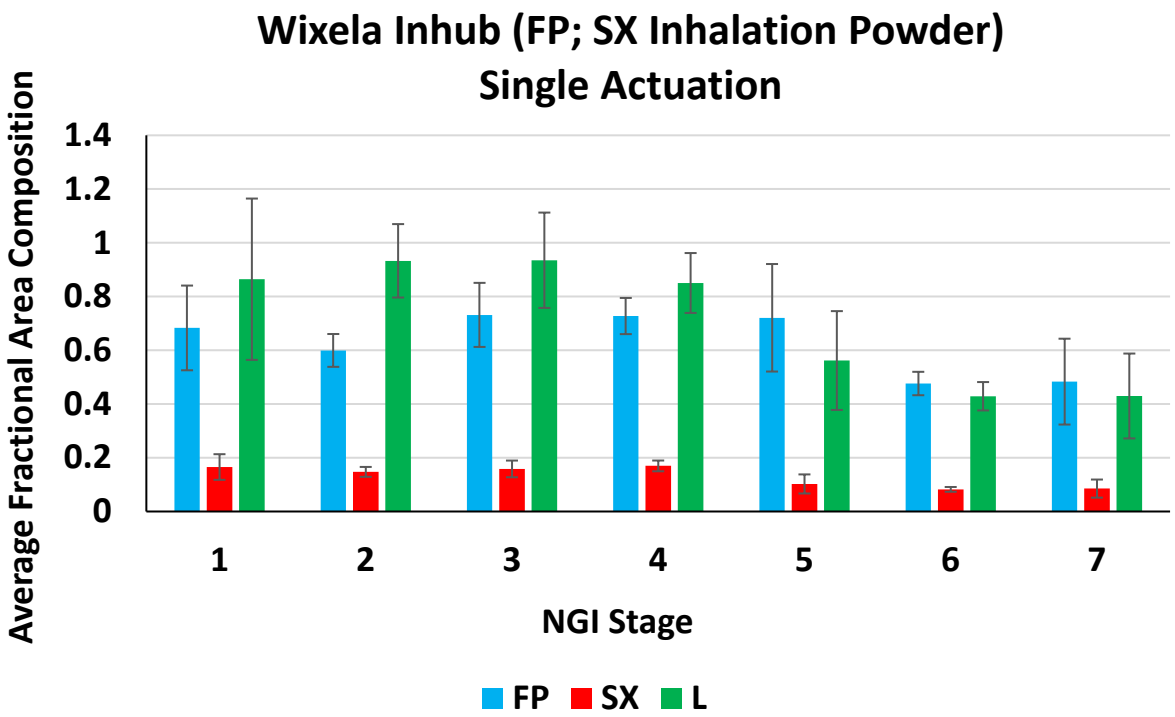
Advair Diskus (FP; SX inhalation powder) and Wixela Inhub (FP; SX inhalation powder) at 0.1 mg/inh; EQ 0.05 mg base/inh strength show similar total material deposited on the CaF_2 surface as measured by total surface area of material

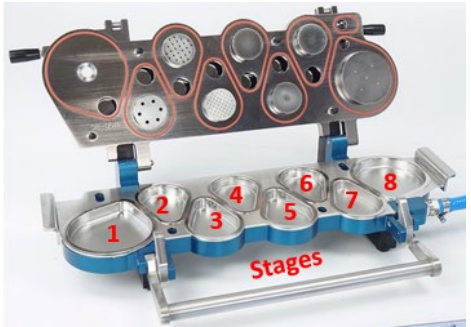
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Average Fractional Area Composition of Particles on CaF_2 Surface

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)





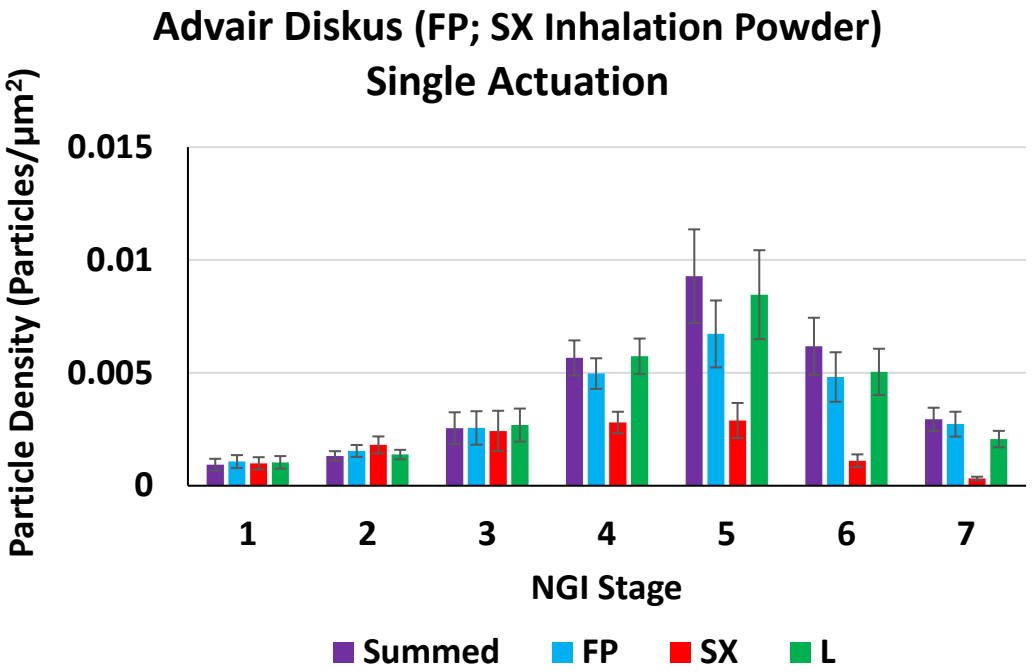


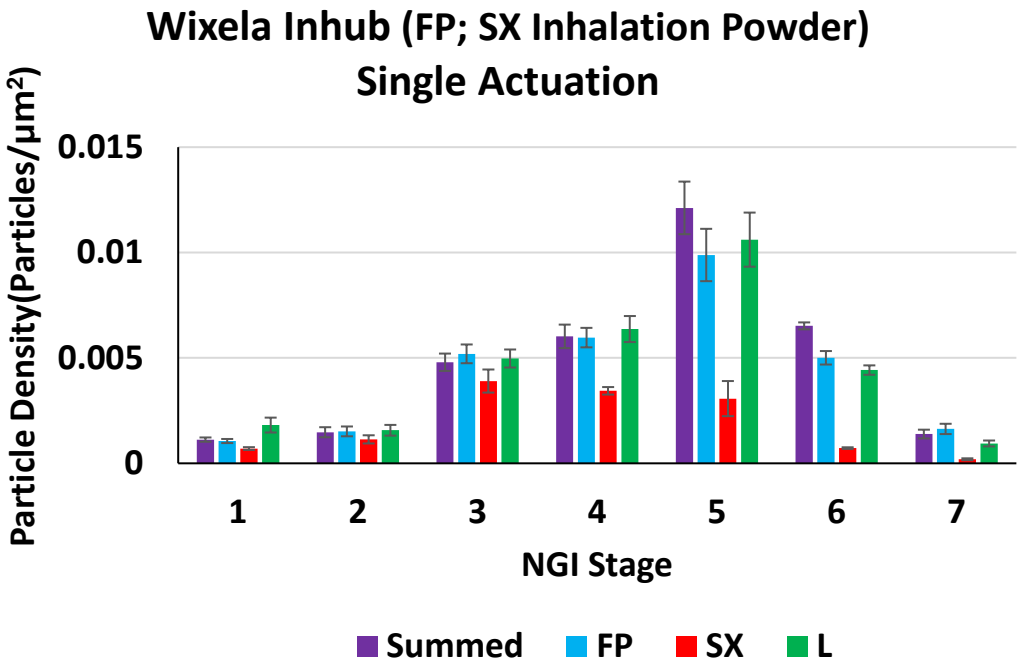
FP; SX inhalation powders, Advair Diskus and Wixela Inhub, at 0.1 mg/inh; EQ 0.05 mg base/inh strength are quite similar in fractional composition of the particles for both Fluticasone and Lactose

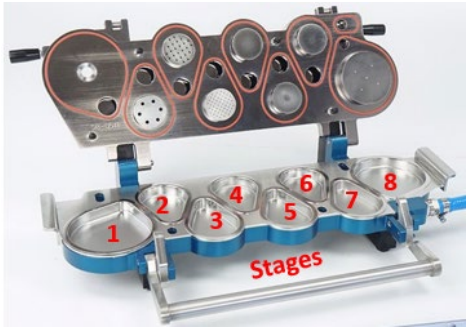
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Density of Particles on CaF_2 Surface

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)



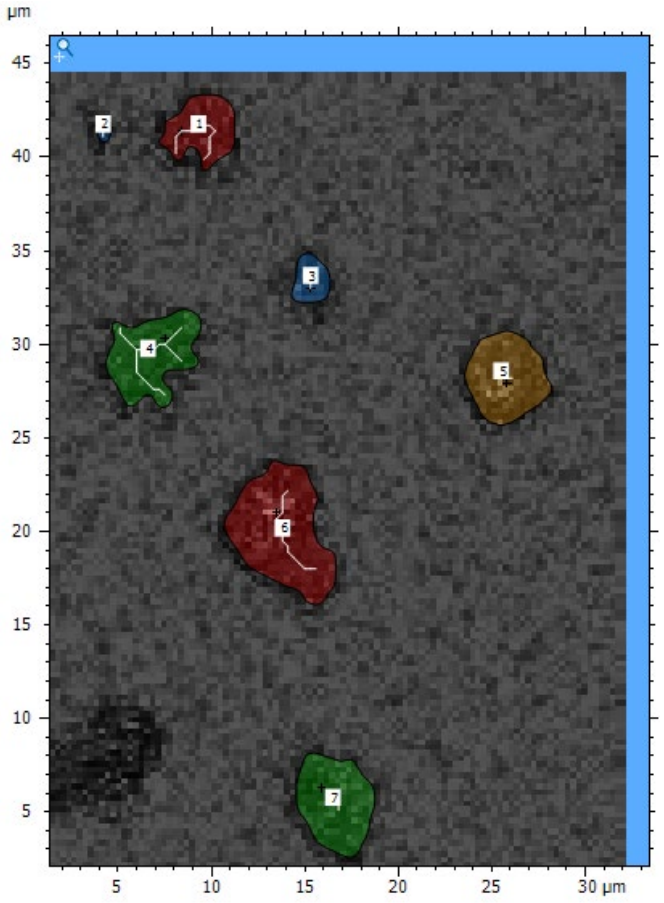




FP; SX inhalation powders, Advair Diskus and Wixela Inhub, at 0.1 mg/inh; EQ 0.05 mg base/inh strength, show similar trend in particle density as a function of NGI stage

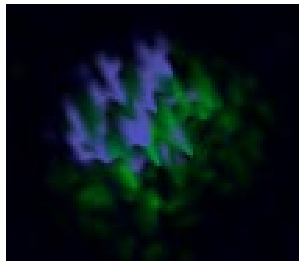
FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Definitions of Particle Area and Diameter



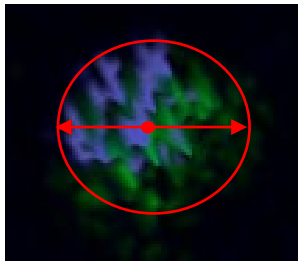
Area and Diameter Analysis for 7 Particle Case (1662 cm^{-1} image of Advair Diskus [FP; SX inhalation powder])

Spectroscopic Area



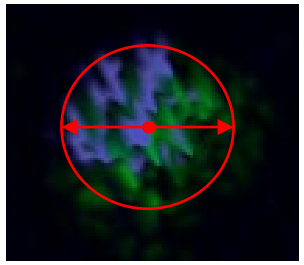
O-PTIR Single Wavenumber Defined Region

Spectroscopic Equivalent Area Diameter



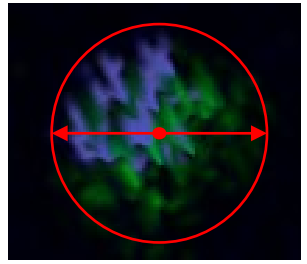
Diameter circle of equiv. area of particle

Spectroscopic Mean Diameter



Diameter defined by particle center of gravity

Spectroscopic Maximum Diameter



Diameter defined by longest rotation axis.

Individual results

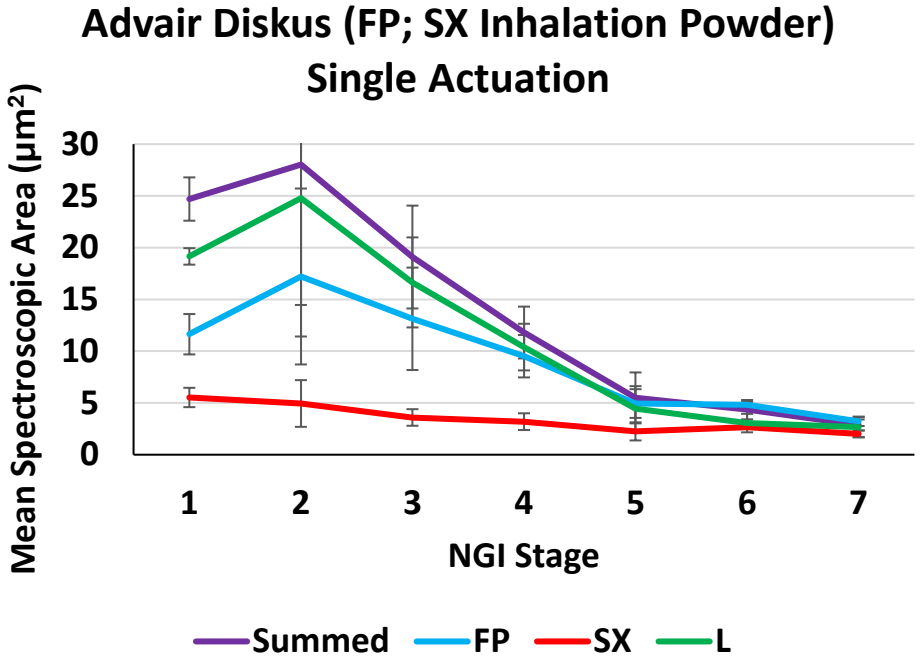
Parameters	Area	Equivalent diameter	Mean diameter	Maximum diameter
Unit	µm²	µm	µm	µm
Particle #1	10.78	3.705	3.525	4.216
Particle #2	0.8480	1.039	0.8963	1.315
Particle #3	4.150	2.299	2.157	2.645
Particle #4	17.30	4.694	4.502	5.787
Particle #5	16.41	4.571	4.404	5.023
Particle #6	27.92	5.962	5.739	7.568
Particle #7	17.13	4.670	4.503	5.859

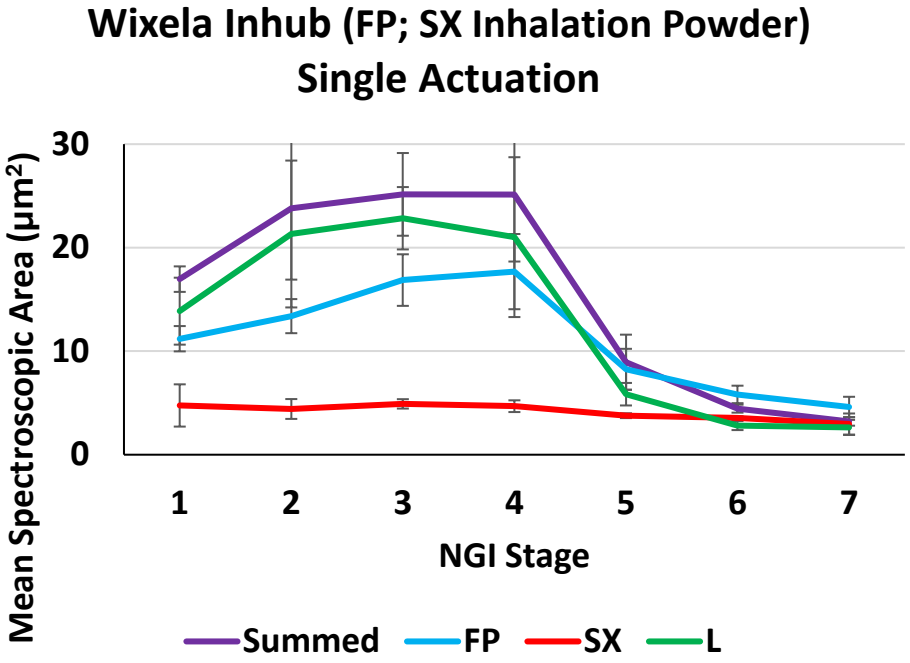
Global statistics

Mean	13.51	3.849	3.675	4.630

Mean Particle Area as Function of NGI Stage

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

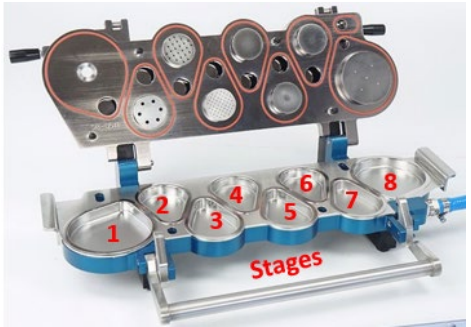


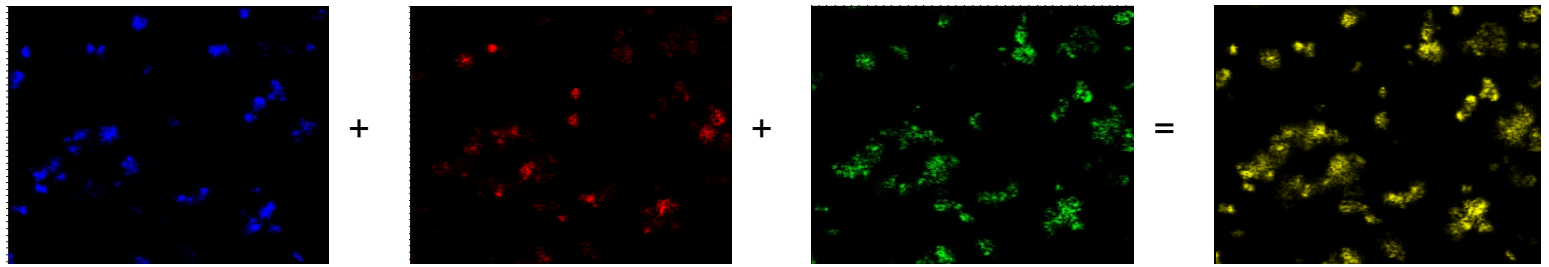


Aggregate particle size decreases with NGI Stage

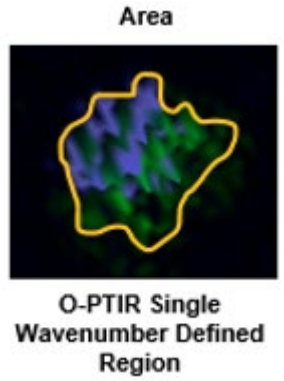
Fluticasone and Lactose also decrease with NGI Stage

Salmeterol particle size constant!





FP SX L Summed

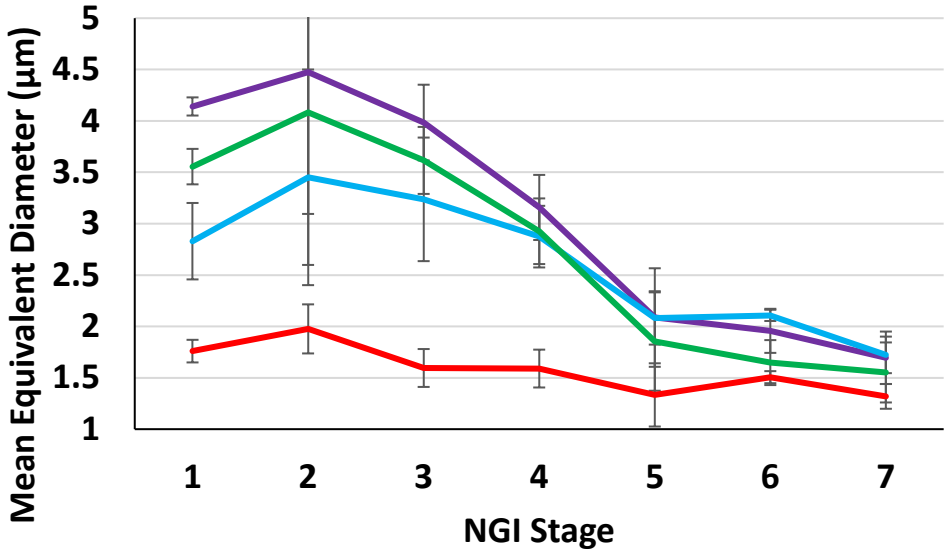


FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

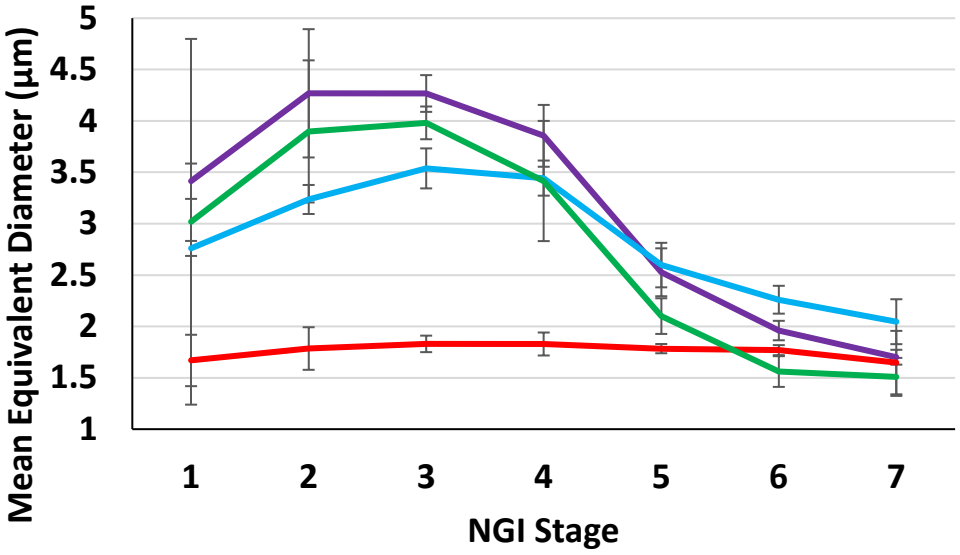
Mean Particle Equivalent Area Diameter as Function of NGI Stage

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

Advair Diskus (FP; SX Inhalation Powder)
Single Actuation



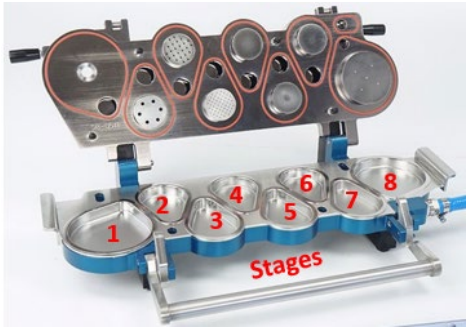
Wixela Inhub (FP; SX Inhalation Powder)
Single Actuation

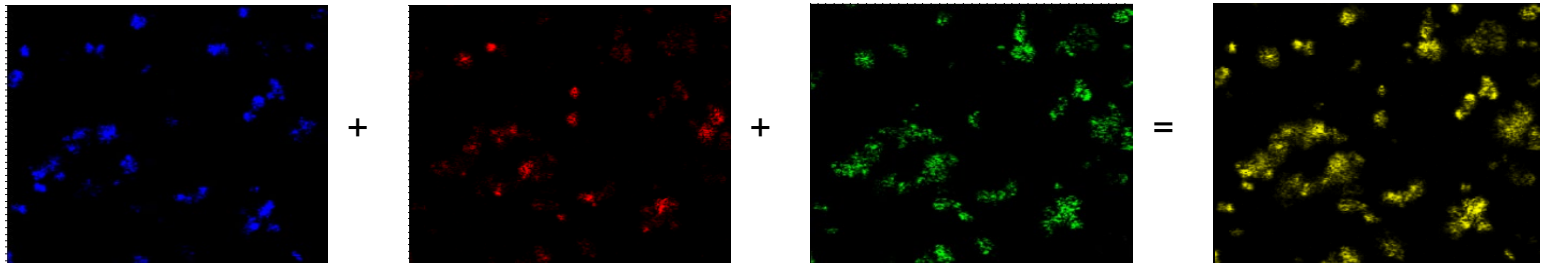


Aggregate particle size decreases with NGI Stage

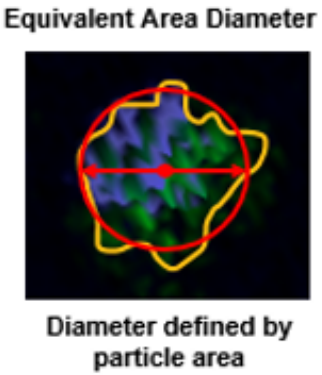
Fluticasone and Lactose also decrease with NGI Stage

Salmeterol particle diameter is constant!





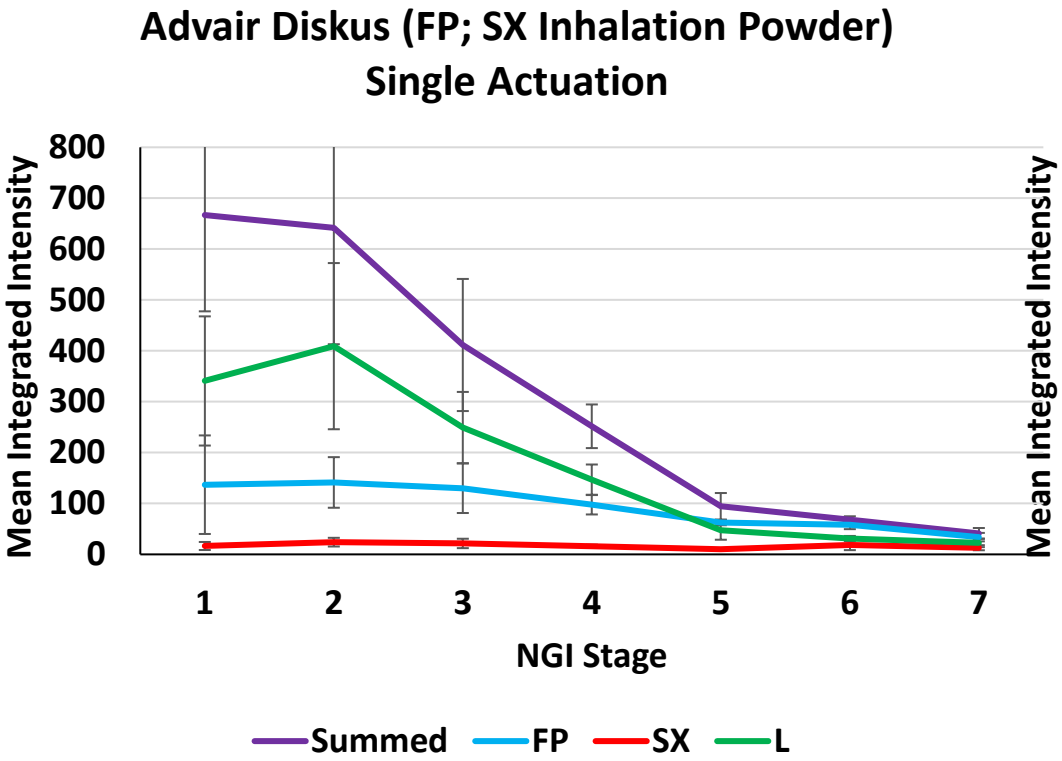
FP SX L Summed

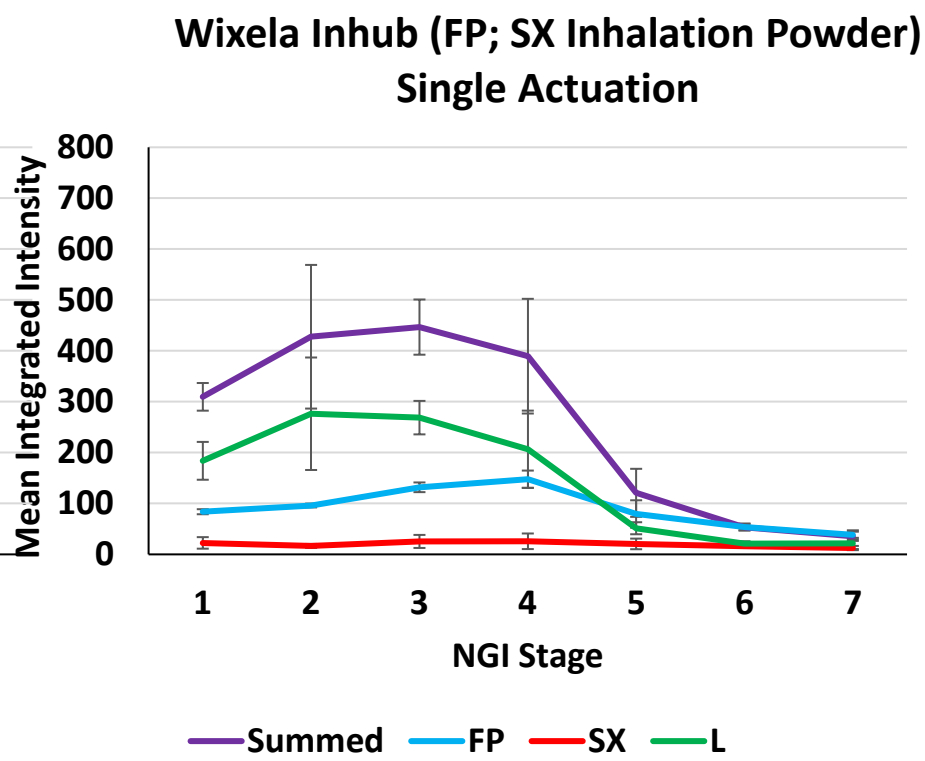


FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Mean Integrated Intensity as Function of NGI Stage

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

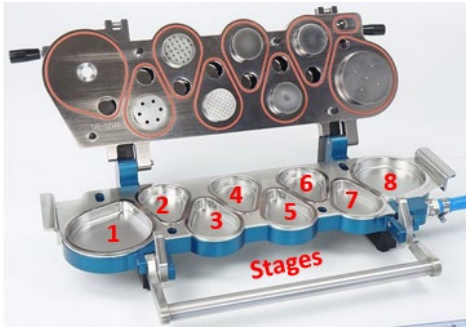


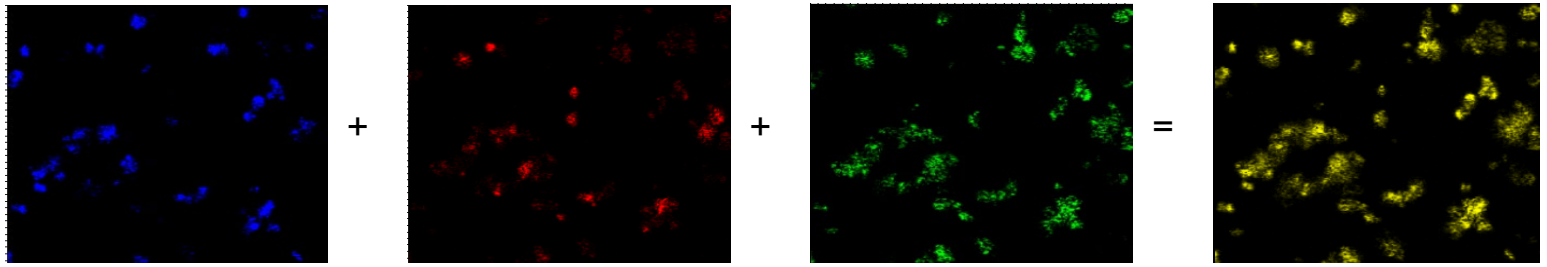


Aggregate particle volume decreases with NGI Stage. AD volume appears larger than WI for Stages 1-3.

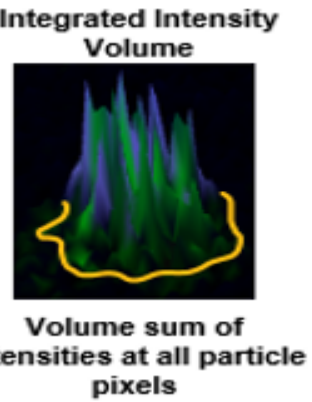
Lactose and Fluticasone volume decrease with NGI stage

Salmeterol particle volume is constant!





FP SX L Summed

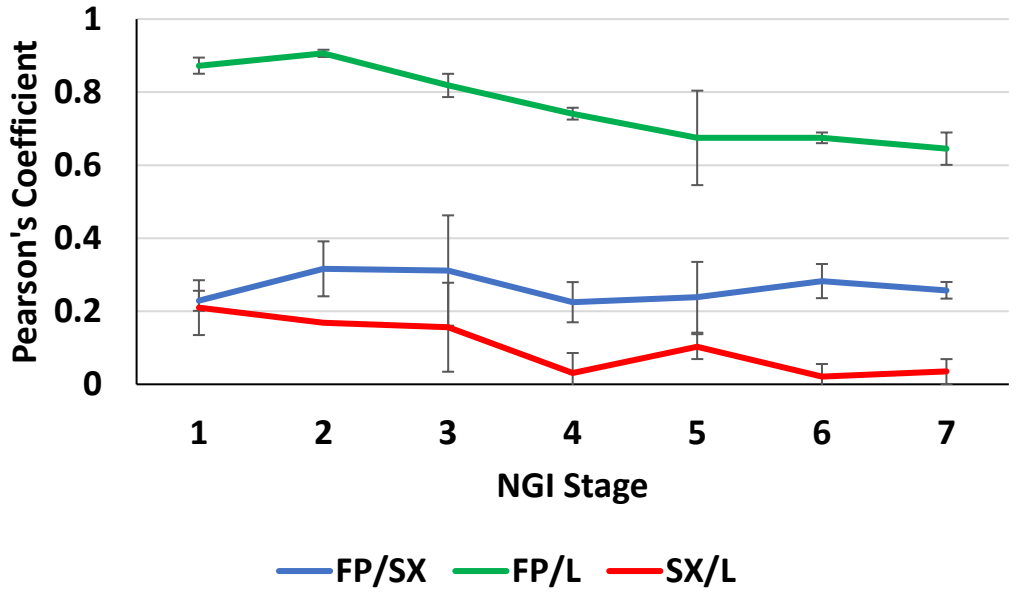


FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

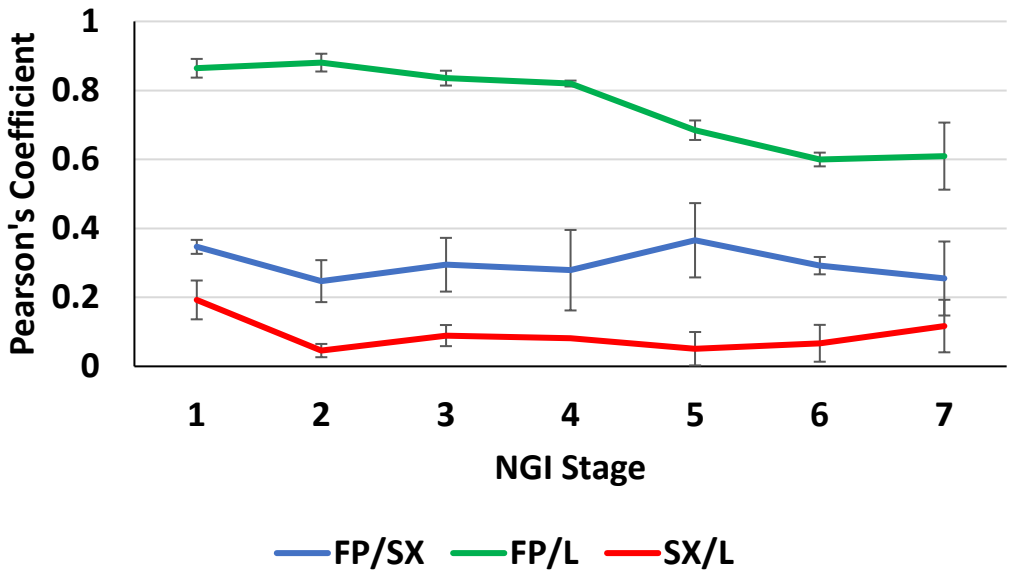
Pearson Coefficient Colocalization Analysis of FP; SX Inhalation Powders, Advair Diskus and Wixela Inhub, as function of NGI stages 1-7

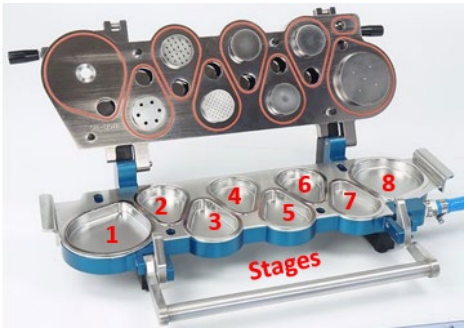
Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

Advair Diskus (FP; SX Inhalation Powder) Single Actuation



Wixela Inhub (FP; SX Inhalation Powder) Single Actuation





The Pearson correlation coefficient indicates the **strength and direction** of a linear relationship between two continuous variables. +1 Positive Linear Correlation, -1 negative linear correlation, 0 no correlation

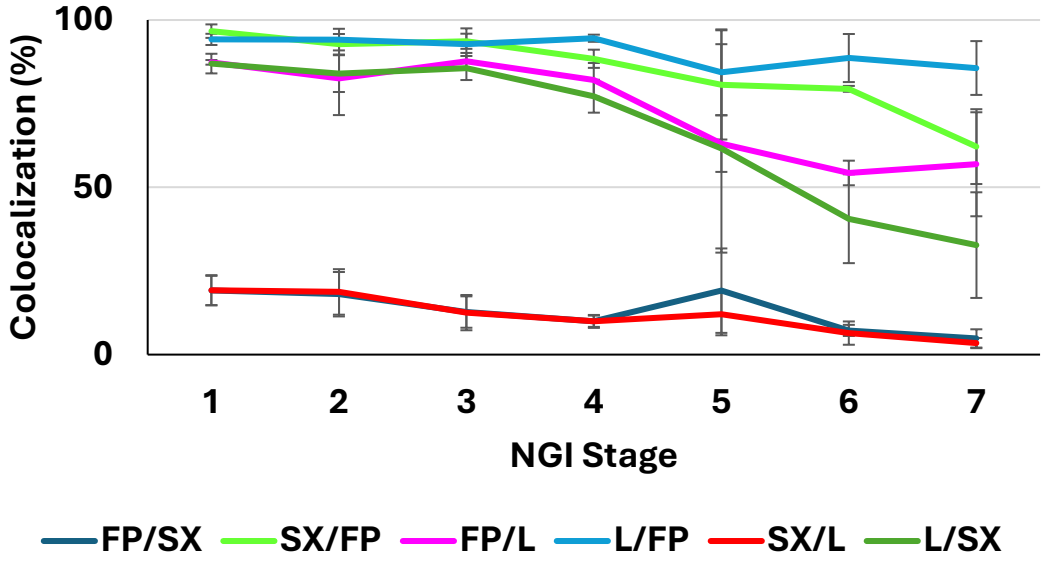
- FP and L exhibit a strong, positive correlation for both formulations
- FP and SX exhibit a weak, positive correlation for both formulations
- There is little correlation SX and L

FP; SX inhalation powders, Advair Diskus and Wixela Inhub (0.1 mg/inh; EQ 0.05 mg base/inh), show similar drug/drug and drug/excipient colocalization properties.

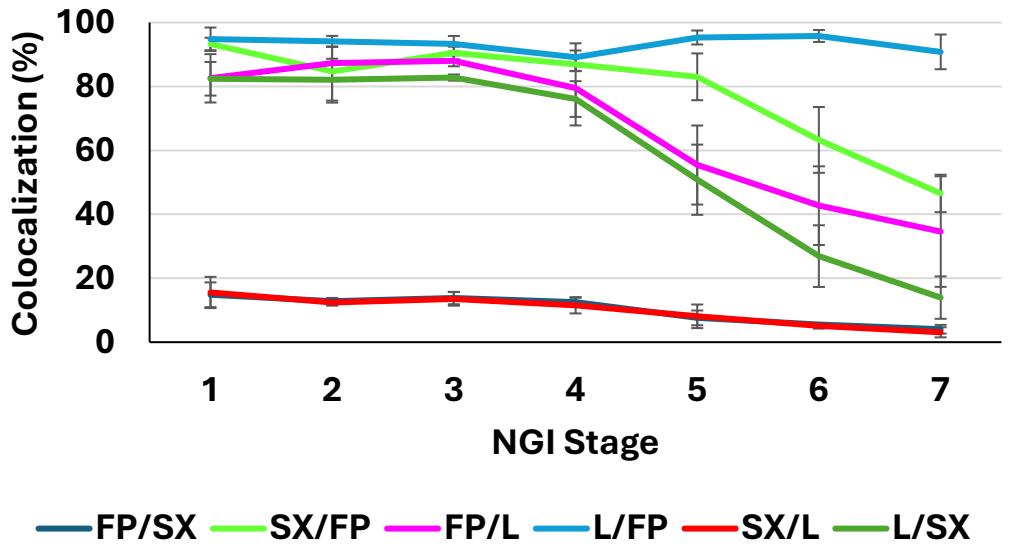
Manders Coefficient Colocalization Analysis of FP; SX Inhalation Powders, Advair Diskus and Wixela Inhub, as function of NGI stages 1-7

Strength: 0.1 mg/inh; EQ 0.05 mg base/inh (FP; SX)

Advair Diskus (FP; SX Inhalation Powder) Single Actuation



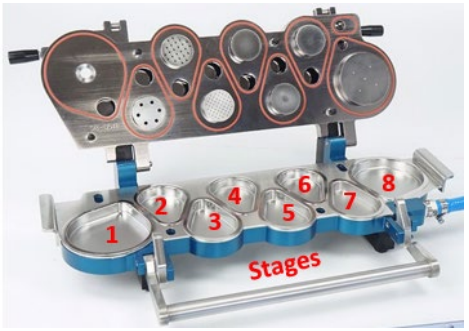
Wixela Inhub (FP; SX Inhalation Powder) Single Actuation



FP/SX SX/FP FP/L L/FP SX/L L/SX

FP/SX SX/FP FP/L L/FP SX/L L/SX

The Manders correlation coefficient indicates the degree of spatial colocalization between a pair of Infrared signals. A value of 1 indicates complete colocalization and a value of 0 indicates no colocalization.

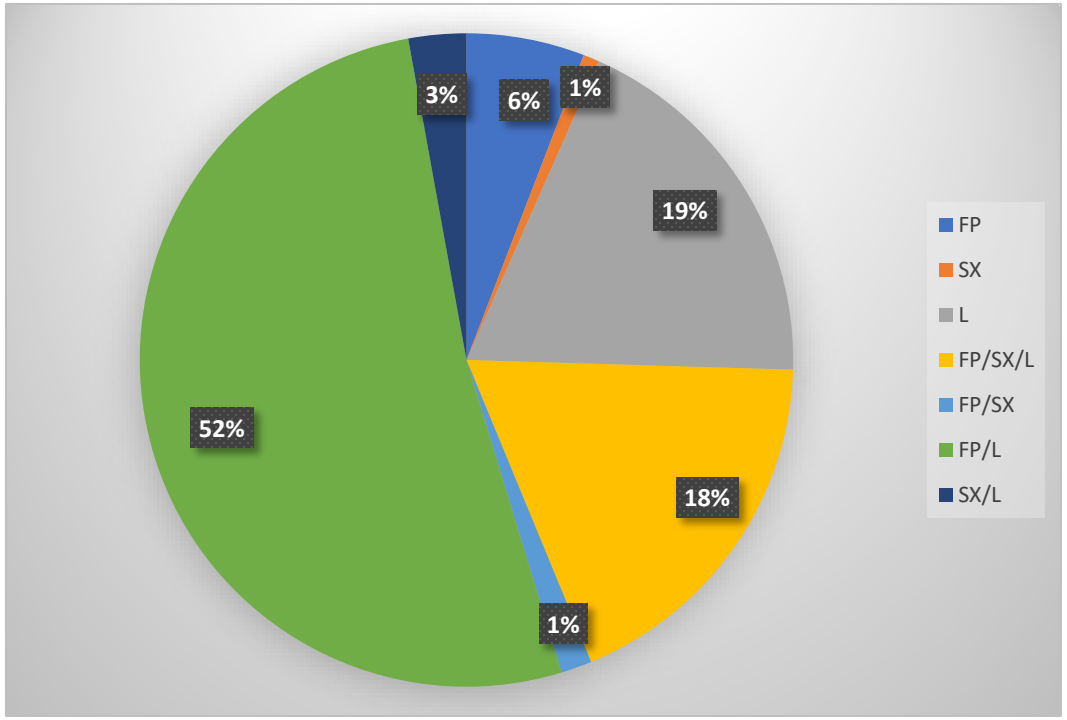


- L is highly spatially localized with FP
- FP is highly spatially localized with L for larger particles and less so for smaller particles
- SX is highly spatially localized with FP
- FP is not spatially localized with SX (there is far more FP in formulation)
- SX is not spatially localized with L

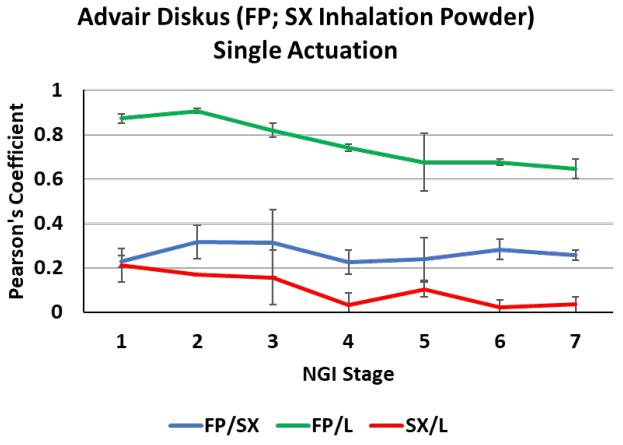
FP; SX inhalation powders, Advair Diskus and Wixela Inhub (0.1 mg/inh; EQ 0.05 mg base/inh) show similar drug/drug and drug/excipient spatial colocalization properties

MDRS and O-PTIR Comparison: NGI Stage 2

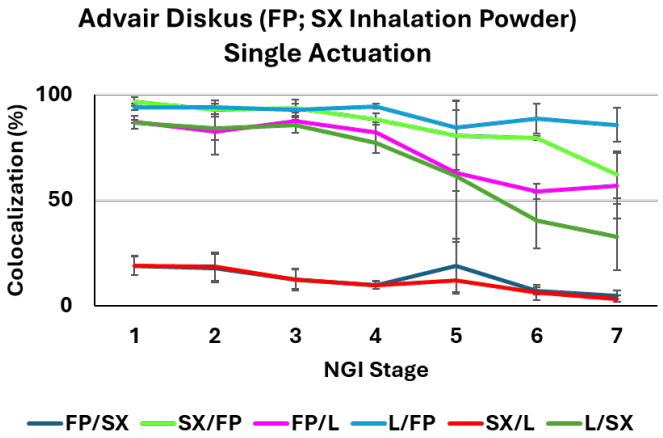
Single Actuation, NGI Stage 2



Pearson Coefficient



Manders Coefficient



Consistency:

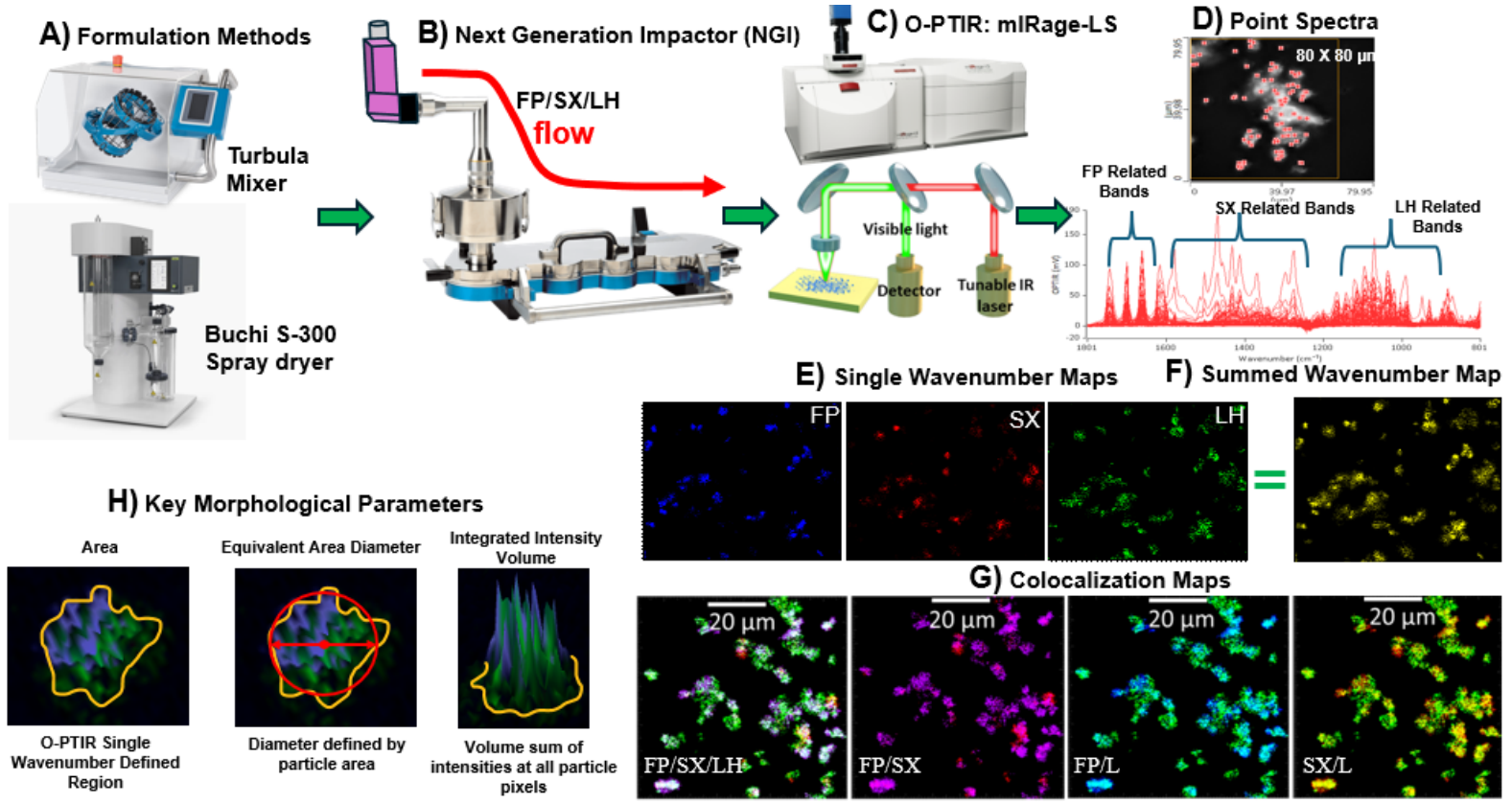
FP/L as major component of aggregates
SX/L as minor component of aggregates
FP/SX as minor component of aggregates

Note that O-PTIR also indicates that SX most often accompanied by FP consistent with Mass Spectroscopy reports

FP: fluticasone propionate; SX: salmeterol xinafoate; L: lactose monohydrate

Farias G, Ganley WJ, Price R, Conti DS, Mangal S, Bielski E, Newman B, Shur J., *Pharmaceutical Research*. **2024** Oct;41(10):2015-29.

O-PTIR with Quantitative Morphology Analysis



- Quantitative evaluation of area and diameter at the particle level for every component and the aggregate particles
- Quantitative evaluation of component colocalization
- Detailed morphological comparison of Brand Name and Generic Formulations

All for a Single Inhaler Actuation and Measured on Lung Relevant Emitted Dose