Alternative Bioequivalence Approach Using Morphologically-Directed Raman Spectroscopy (MDRS) on Nasal Spray Suspensions

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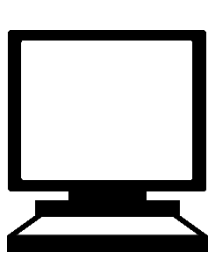
Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



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Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.

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Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.


It is what gives patients confidence
in their *next* dose of medicine.

Outline

- Introduction to Morphologically Directed Raman Spectroscopy (MDRS)
  - Optical Microscopy
  - Raman Spectroscopy
  - Morphological characterization

- Example of utilizing MDRS on nasal spray suspension
  - Sample preparation
  - Image and particle analysis
  - Morphology screening and filter selection

Why MDRS?

Particle size distribution of active pharmaceutical ingredient (API) **in the drug product** is a critical attribute in evaluating complex drug products

- Quality
- Effectiveness
- Bioequivalence (BE) (for evaluating generic drugs)

Challenges:

- **API and excipient particles coexist in the formulation**
- **More than one APIs in the formulation**
- **API may have more than one polymorphic form**

Traditional particle sizing techniques, such as cascade impaction, laser diffraction, and microscopy cannot distinguish particles with different chemical identities.

Microscopy and Raman Spectroscopy

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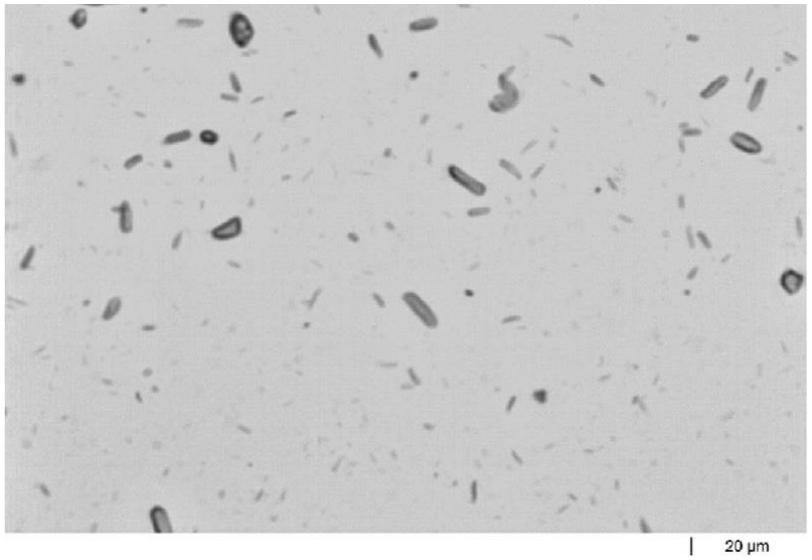
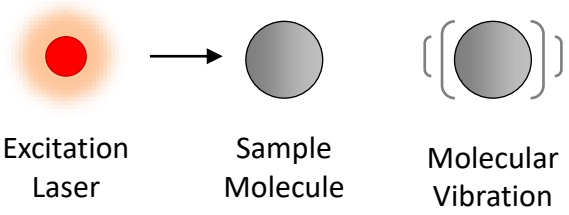
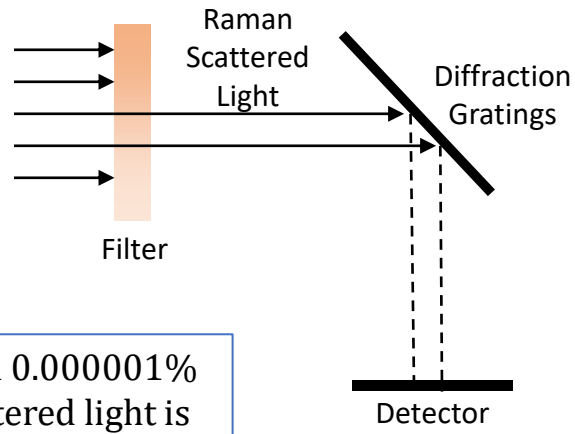
Microscopy



Raman Spectroscopy

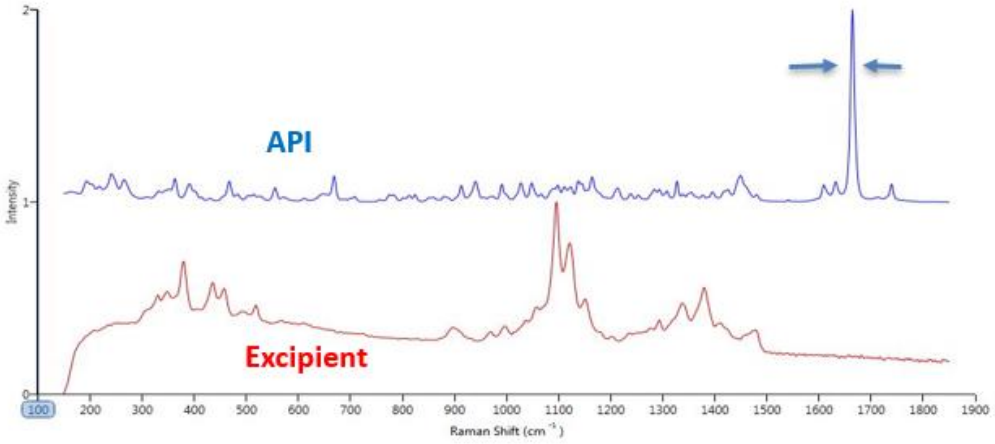
$Incident\ Frequency\ = v_i$





Around 0.000001% of scattered light is Raman scattering

$Scattered\ Frequency\ = v_s \neq v_i$

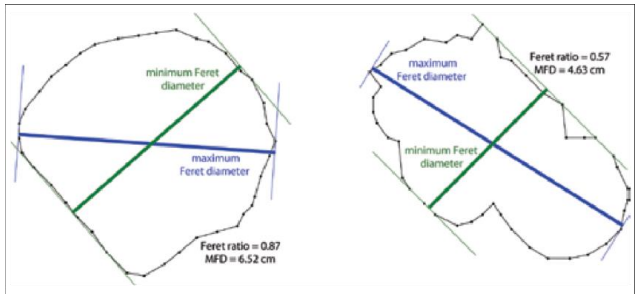


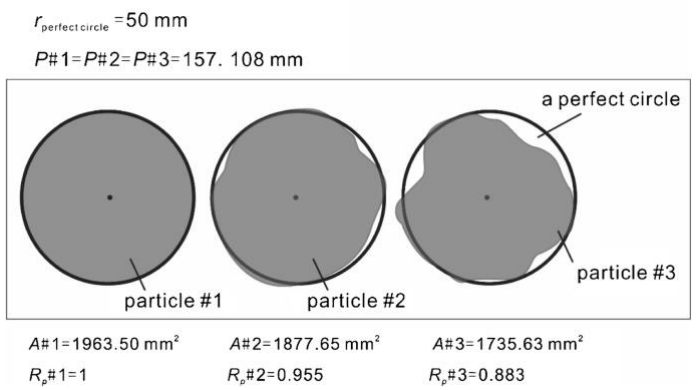
Morphological characterization/screening

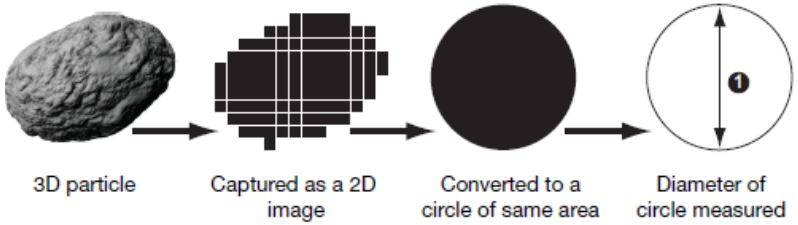
- Particle size represented by **circular equivalent (CE) diameter**: $d = \sqrt{\frac{4A}{\pi}}$
- Particle shape characterized by **aspect ratio** and **circularity**:

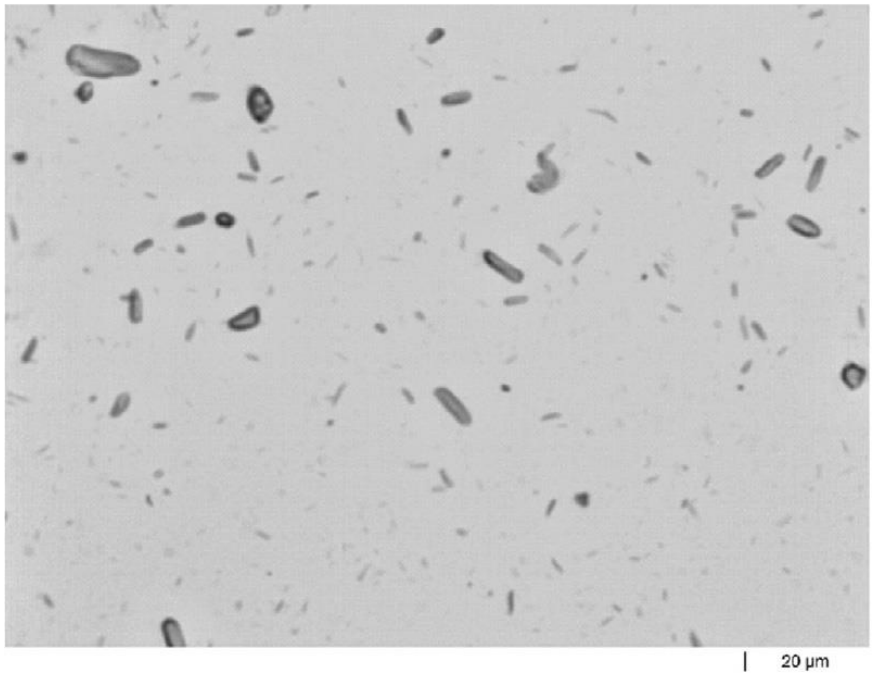
$$AR = \frac{x_{F\,min}}{x_{F\,max}}$$

$$C = \sqrt{\frac{4\pi A}{P^2}}$$





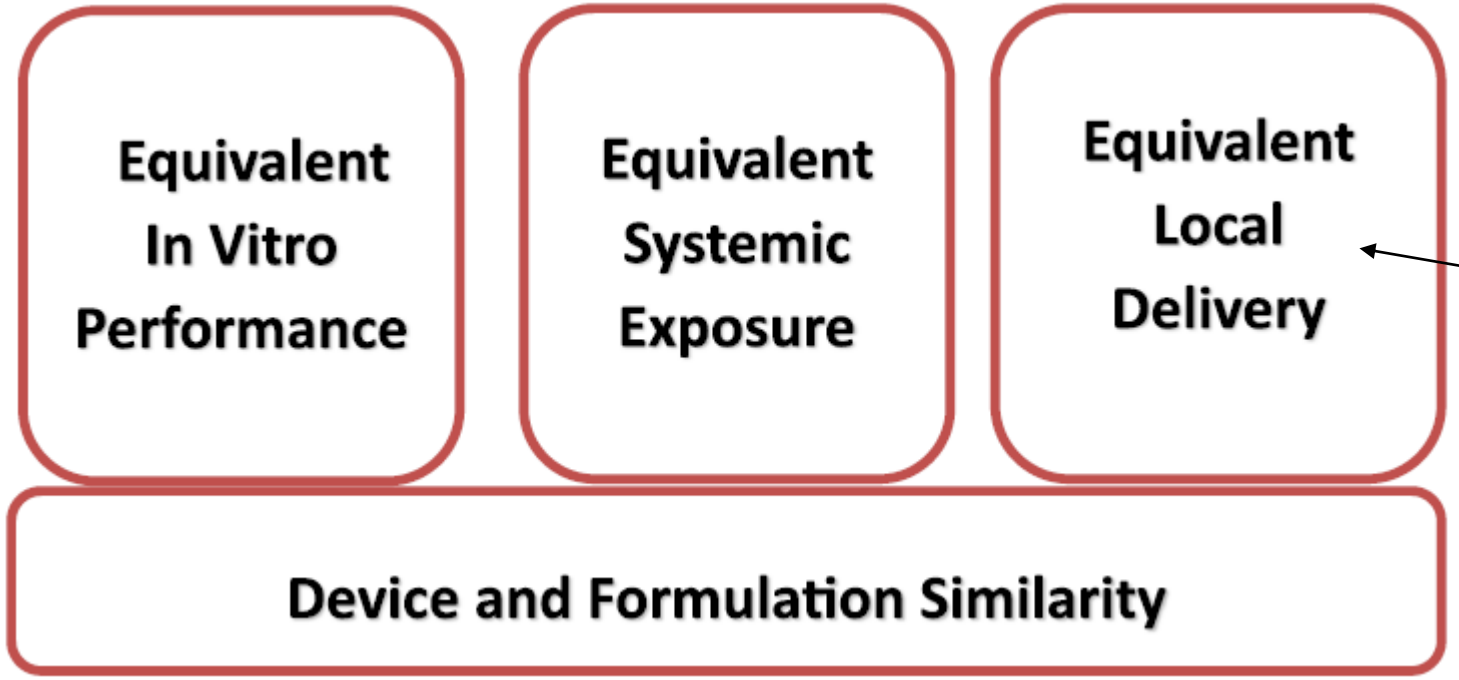




Generic Nasal Spray Suspension

- Establishing bioequivalence of locally-acting drugs can be a challenging task.
- Using traditional pharmacokinetic study results as pivotal evidence to support bioequivalence for the systemically-acting drugs is not applicable to the locally-acting drugs.
- Aerosolized nasal drug products are also integrated with a device; therefore, the interaction between the drug formulation and the delivery device also plays a role in ensuring bioequivalence.

Weight-of-evidence approach



- Comparative in vitro studies
- Comparative pharmacokinetic studies
- Comparative clinical endpoint studies

MDRS Method Development

A five-step method development procedure was used in this study:

1. Sample preparation
2. Particle imaging and morphology analysis
3. Particle Raman measurements and classification
4. Morphology filter selection
5. Minimum number of particles determination

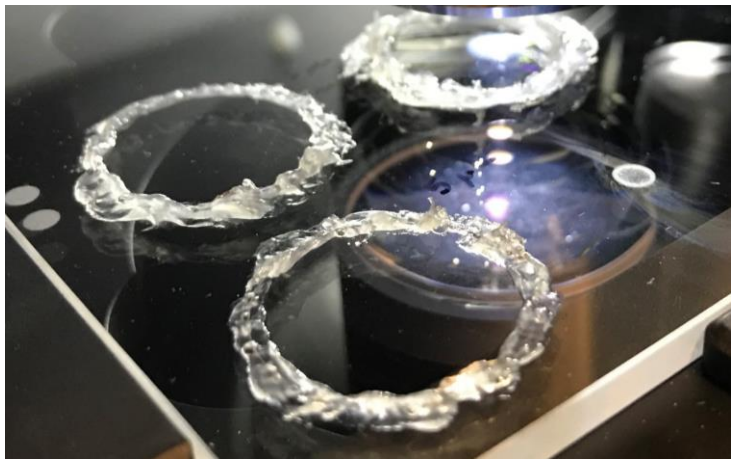
A training set containing over 10,000 randomly selected particles, including both the API and excipient particles, was used to gain a comprehensive understanding of particle size, shape, and chemical ID for the nasal spray suspension.

Sample Preparation

Wet dispersion method

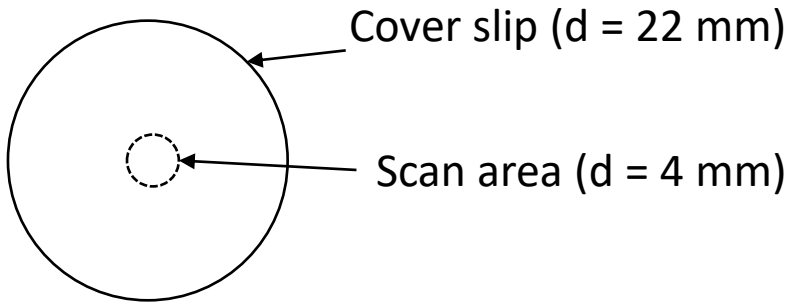
- Dry dispersion would cause particle aggregation upon solvent evaporation.

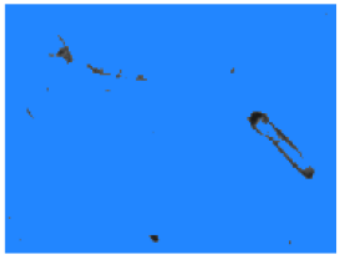
1. Prime nasal spray (2 actuations to waste) and collect 2 actuations into glass vial.
2. Pipette 5 µL onto a quartz microscope slide and cover with quartz coverslip.
3. Seal with petroleum jelly along edge of coverslip to prevent evaporation.
4. Let sample rest for 1 hour for particles to settle before analysis.



Particle imaging and morphology analysis

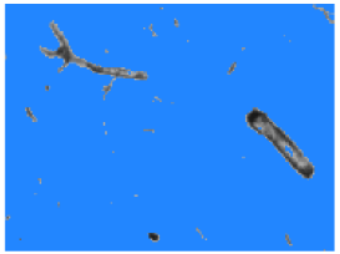
- Scan area with radius = 2 mm
- Collect minimum of 10000 suspended particles
- 50x objective lens, filter particles with circular equivalent (CE) diameter < 1 µm
- Light intensity calibrated to 80.00 ± 0.20%
- Edges of the particles were determined via image binarization with a user defined intensity threshold
- X-Y coordinates of found particles logged.





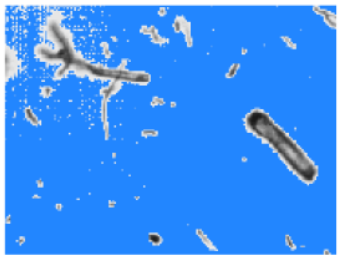
Threshold = 100

Too low, particles begin to disappear.



Threshold = 175

Aim for a thin gray border around the edge of the particles and a complete perimeter.



Threshold = 235

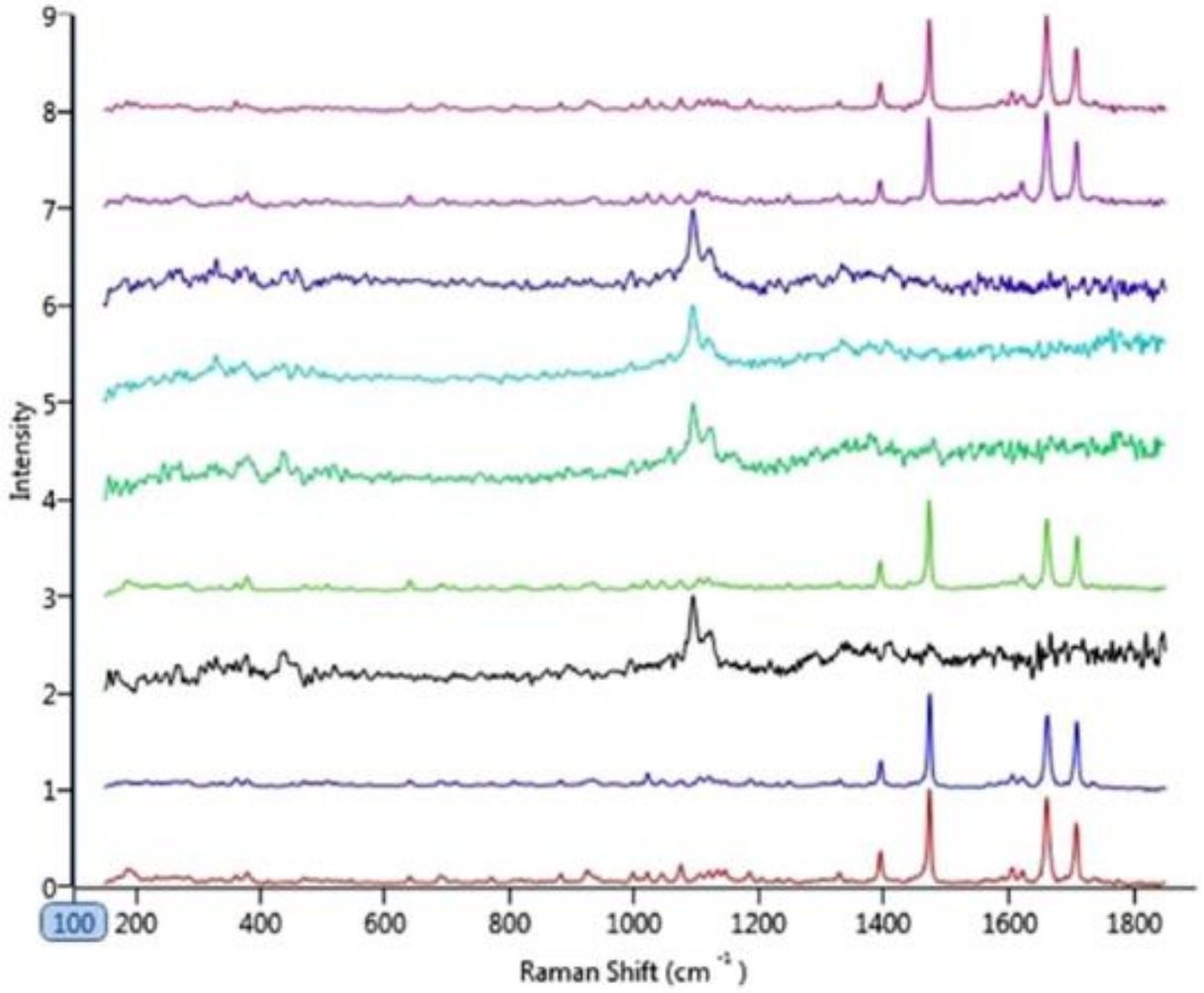
Too high, particles are oversized and the background starts to count.

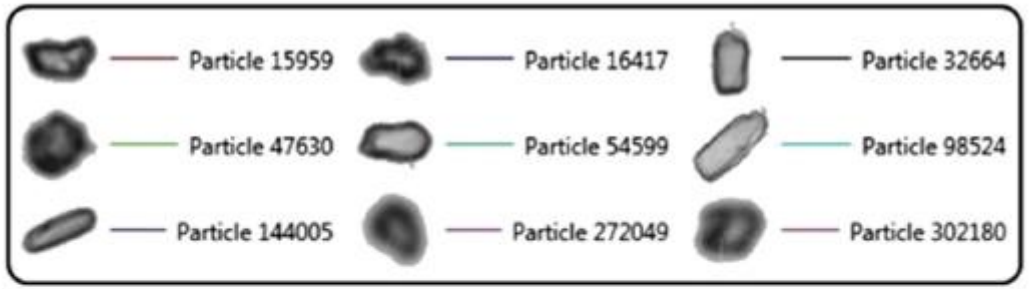
Particle imaging and morphology analysis

Representative images of particles suspended in mometasone furoate nasal formulations. Particle size and morphology data is included for comparison.

Particle #	1	2	3	4	5	6
CE Diameter	2.28 μm	4.69 μm	2.53 μm	8.91 μm	7.36 μm	6.85 μm
Aspect Ratio	0.740	0.460	0.822	0.418	0.709	0.585
Circularity	0.965	0.860	0.974	0.853	0.937	0.795
Intensity Mean	124	155	115	154	107	108
Convexity	0.994	0.974	0.994	0.986	0.988	0.923
Solidity	0.997	0.986	0.998	0.994	0.990	0.886

Raman measurements and classification





Correlations

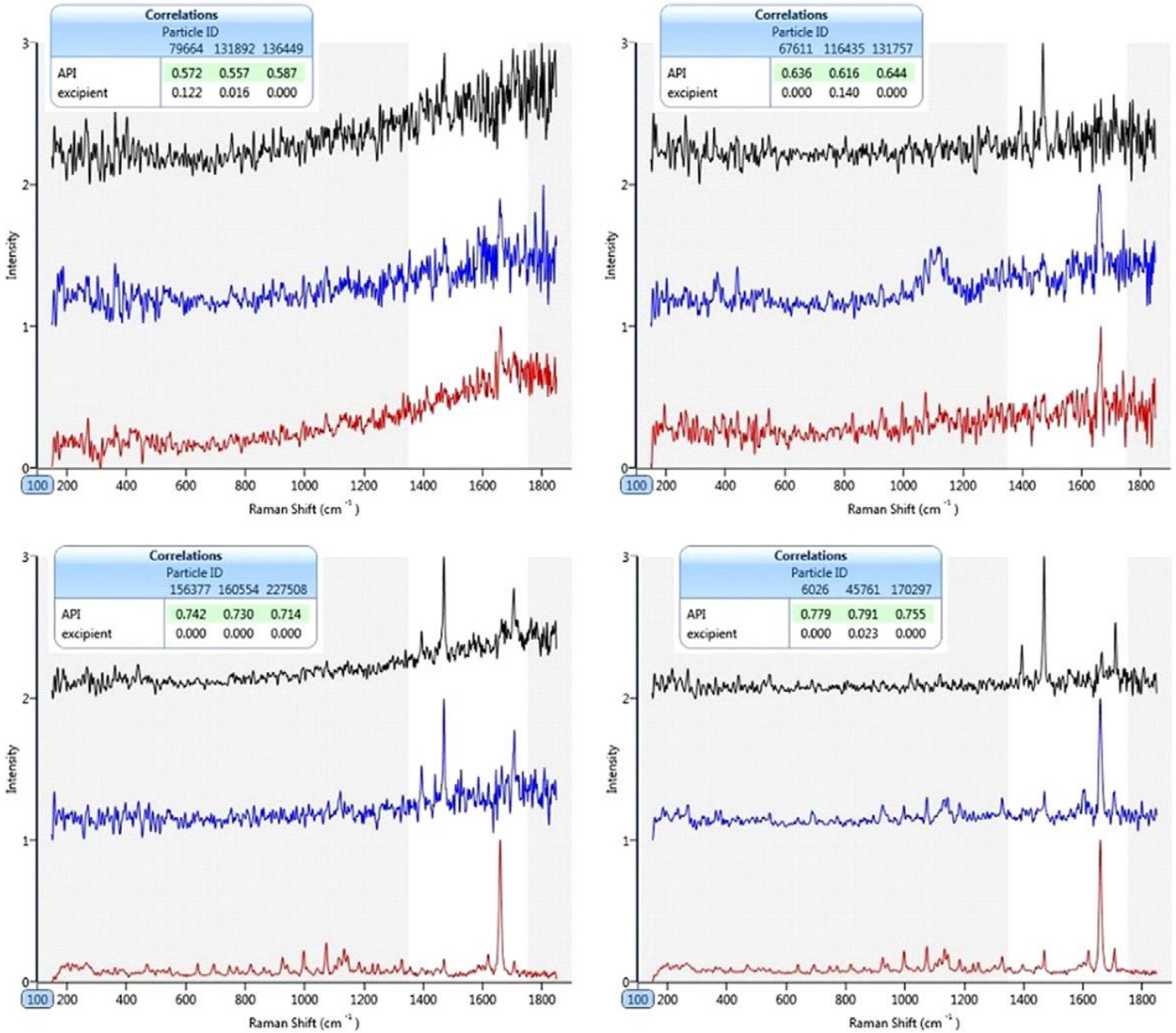
	Particle ID								
	15959	16417	32664	47630	54599	98524	144005	272049	302180
API	0.975	0.980	0.086	0.983	0.052	0.000	0.001	0.976	0.981
excipient	0.000	0.000	0.709	0.000	0.697	0.689	0.707	0.000	0.000

Raman measurements and classification

- Surface roughness could affect the Raman scattering efficiency.

Selection Criteria:

- At least one of the four API signature peaks had to be observed with acceptable S/N (> 3).
- A correlation score > 0.60 was determined for identifying API.



Raman measurements and classification

Training Set Total # Particles Counted = 10835

API particles identified = 1335 (12.3%) Disclaimer: This value is formulation specific!

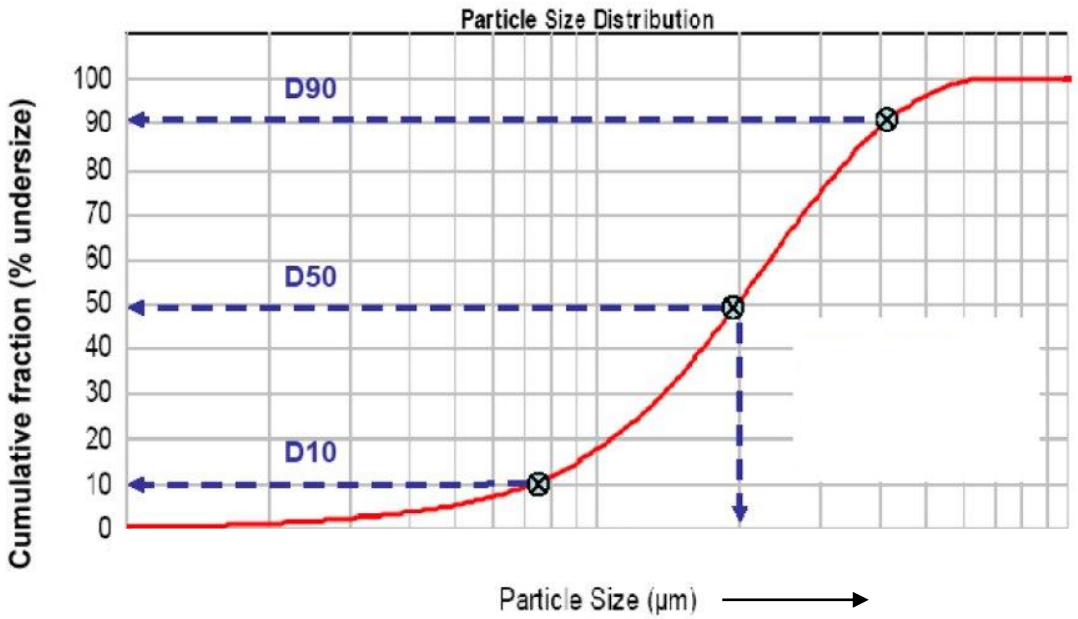
- A typical excipient particle was elongated and transparent
- A typical API particle was close to round or square shape and opaque.

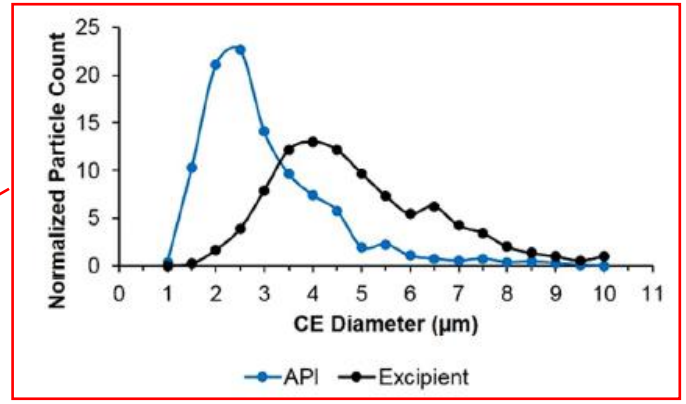
Morphology filter selection

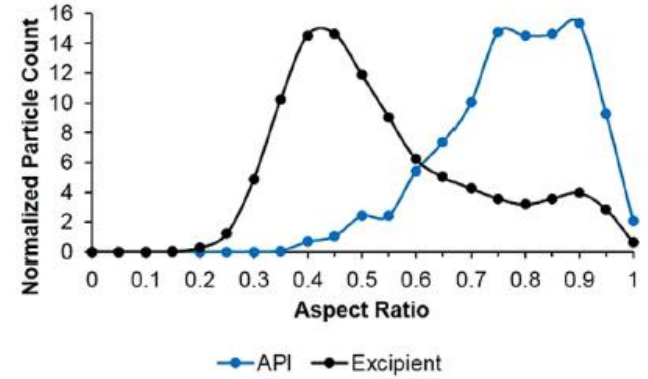
- Raman measurements confirmed separation of API and excipient particles.
  - Problem: 10000 particles would equate to ~90 hours at 20s per collection.
  - Solution: Use morphological screening to reduce excipient Raman detection.

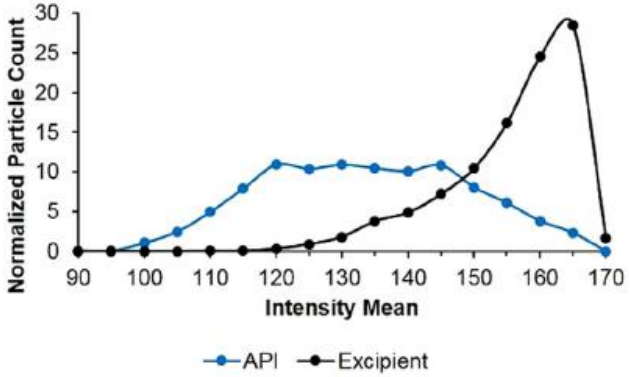
- Morphological screening: choose an appropriate morphology filter set to eliminate as many excipient particles as possible, and at the same time, keep as many API particles as possible without adversely influencing measurement results.
  - Care must be taken to not remove too many of the API particles of interest.

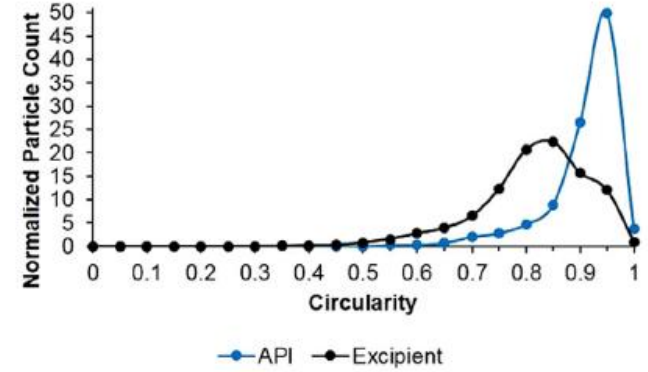
Morphology filter selection

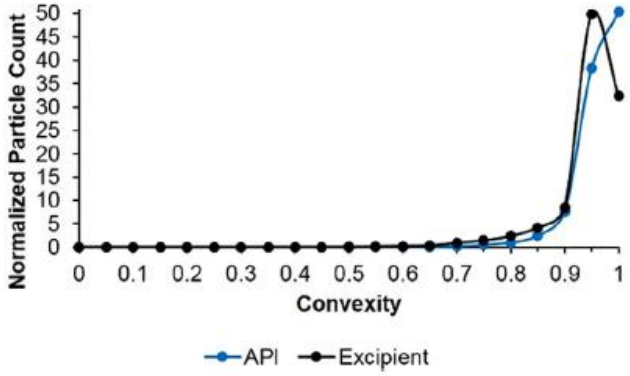


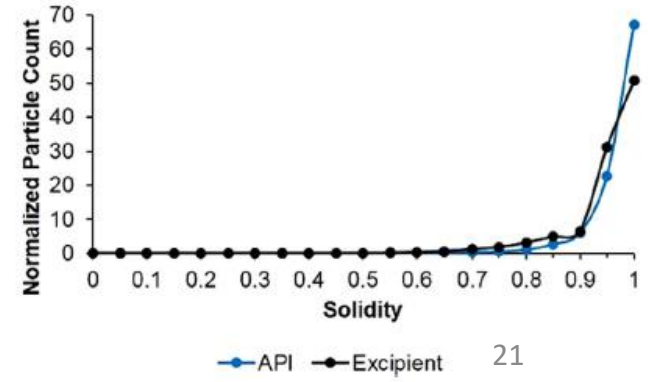












Morphology filter selection

Summary of API and excipient particle counts from a nasal spray sample upon application of aspect ratio (AR) and intensity mean (IM) morphology filters. To meet the criterion for selection, the percentage of API particles retained upon application of the morphology filters must be greater than 85%. Those values meeting the criteria are highlighted in blue, and those not meeting criteria are highlighted in red.

	No Filters	AR > 0.60IM < 150	AR > 0.60IM < 155	AR > 0.60IM < 160	AR > 0.55IM < 155	AR > 0.50IM < 150
API Particle Count	1335	1035	1120	1170	1170	1110
Excipient Particle Count	9500	470	830	1380	1070	840
Total Particle Count	10835	1505	1950	2550	2240	1950
% API Particles	12.3%	68.8%	57.4%	45.9%	52.2%	56.9%
# API Particles Removed	0	300	215	165	165	225
# Excipient Particles Removed	0	9030	8670	8120	8430	8660
% API Particles Retained	100%	77.5%	83.9%	87.6%	87.6%	83.1%
% Excipient Particles Removed	0%	95.0%	91.3%	85.5%	88.7%	91.2%

Summary of API PSD data before and after application of aspect ratio (AR) and intensity mean (IM) morphology filters. The variations before and after applying the filters were shown in parenthesis. To meet the criterion for selection, the variations in D10, D50, D90, and Dmean should be no more than 3%. Those values not meeting criteria are highlighted in red.

	No Filters	AR > 0.60IM < 150	AR > 0.60IM < 155	AR > 0.60IM < 160	AR > 0.55IM < 155	AR > 0.50IM < 150
D_{mean} (μm)	3.00	3.10 (3.3%)	3.03 (1.0%)	2.98 (0.7%)	3.06 (2.0%)	3.15 (5.0%)
D10 (μm)	1.71	1.90 (11%)	1.83 (7.0%)	1.75 (2.3%)	1.84 (7.6%)	1.91 (12%)
D50 (μm)	2.60	2.69 (3.5%)	2.61 (0.4%)	2.56 (1.5%)	2.64 (1.5%)	2.73 (5.0%)
D90 (μm)	4.60	4.63 (0.7%)	4.60 (0.0%)	4.54 (1.3%)	4.61 (0.2%)	4.67 (1.5%)
Span	1.11	1.01 (9.0%)	1.06 (4.5%)	1.09 (1.8%)	1.05 (5.4%)	1.01 (9.0%)

- Filters reduce measurement time from 90 hours to 22 hours.

Determination of minimum number of particles

Used computational sampling to mimic in vitro characterization (n=5).

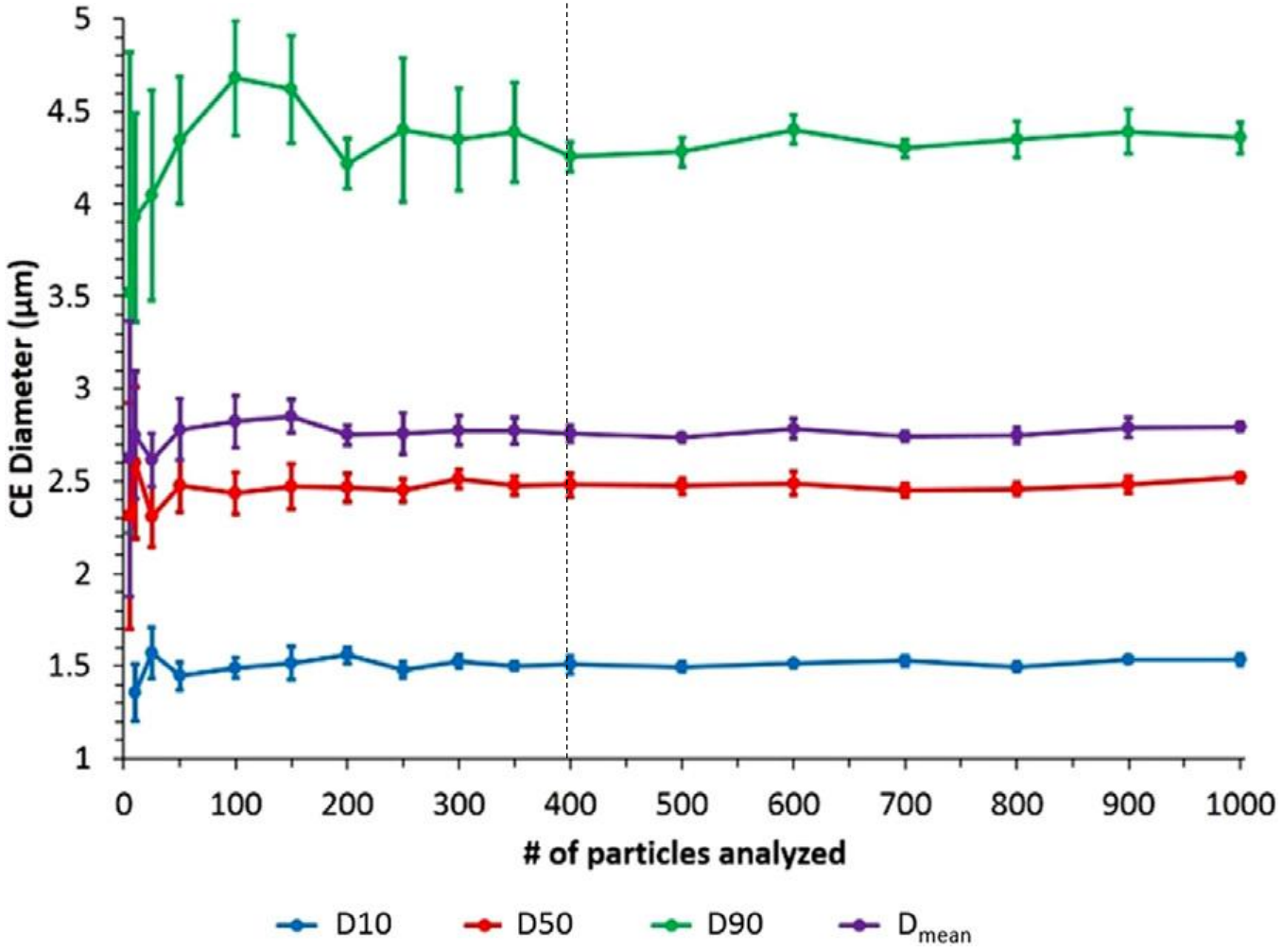
Criteria for accuracy:

- 3% difference from the true values

Criteria for repeatability:

- %RSD of the 5 replicates must be < 5%

Minimum count = 400



Review

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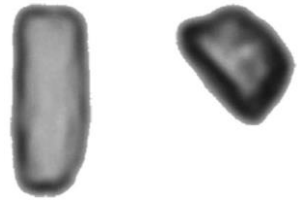
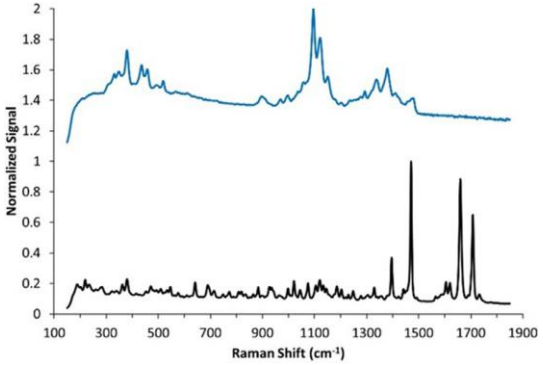
MDRS is an automated microscopy and Raman spectroscopy setup

- Can identify and characterize complex API formulations
- Used for establishing bioequivalence in generic nasal spray suspensions.

Example of using MDRS on nasal sprays

1. Sample preparation - wet dispersion
2. Particle imaging and morphology analysis
3. Particle Raman measurements and classification
4. Morphology filter selection – aspect ratio > 0.6, intensity mean < 160
5. Minimum number of particles determination = ~400 particles





MDRS Product Specific Guidances

Nearly all **suspension nasal spray products** have the language for an "Alternate approach to the comparative clinical endpoint BE study", where MDRS is suggested as one of the techniques.

- Draft Guidance on Mometasone Furoate Monohydrate. 06/2020
- Draft Guidance on Fluticasone Propionate. 06/2020
- Draft Guidance on Triamcinolone Acetonide. 06/2020
- Draft Guidance on Budesonide. 08/2020
- Draft Guidance on Fluticasone Furoate. 06/2020
- Draft Guidance on Azelastine Hydrochloride. 06/2020

FDA Publications

(1) **Scientific Considerations for the Review and Approval of First Generic Mometasone Furoate Nasal Suspension Spray in the United States from the Bioequivalence Perspective**

Qing Liu,[1] Mohammad Absar,[1,2] Bhawana Saluja,[1,2] Changning Guo,[3] Badrul Chowdhury,[4,5] Robert Lionberger,[1] Dale P. Conner,[1] and Bing V. Li[1,6]

(2) Analytical method development for characterizing ingredient-specific particle size distributions of nasal spray suspension products

Brandon J. Thomas[a,1], Mohammad Absar[b,2], Renishkumar Delvadia[b,3], Denise S. Conti[b], Kimberly Witzmann[b,4], Changning Guo[a,*]

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[b] *Office of Research and Standards, Office of Generic Drugs, Food and Drug Administration, Silver Spring, MD, United States*