



BIOBRIDGES 2026

J-M. Cardot

Sept 2026

NASAL SUSPENSION IN VITRO ASSESSMENT

Training materials prepared by J-M. Cardot
© J-M Cardot otherwise mentioned.

Private & Confidential

INTRODUCTION: MOVING AREA

EP REVISED MONOGRAPHS

November 2025, European Pharmacopoeia including an Abbreviated Impactor apparatus together with associated procedure(s) in general chapter *2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles*. The Abbreviated Impactor apparatus can be used to assess the fine particles in inhalation preparations and/or the mass of particles smaller than 10 µm in nasal preparations.

EP REVISED MONOGRAPHS

The Ph. Eur. monograph on *Nasal preparations (0676)* will also be revised to include a requirement to test the uniformity of delivered doses for nasal preparations that are supplied in single-dose containers.

Elaboration of a new general chapter on the assessment of the mass of particles/droplets smaller than 10 µm in nasal preparations (2.9.56) is ongoing.

EP REVISED MONOGRAPHS

Description of standardized equipment and the testing procedure ongoing. The new text will describe a specifically designed inlet for nasal preparations and reference equipment from updated general chapter *2.9.18*.

Covers for Inhalation products and Nasal products

- Active substance (CTD 3.2.S)
- Finished medicinal product (CTD 3.2.P)
- Description and composition of the finished medicinal product (CTD 3.2.P.1)
- Pharmaceutical development (CTD 3.2.P.2)
- Manufacture (CTD 3.2.P.3)
- Control of excipients (CTD 3.2.P.4)
- Control of the finished medicinal product (CTD 3.2.P.5)
- Container Closure System (CTD 3.2.P.7, 3.2.R)
- Stability (CTD 3.2.P.8)
- Therapeutic equivalence
- Product information
- Lifecycle management

STEPWISE APPROACH FOR GENERICS

Q1-Q2 Qualitative and quantitative formulation

Specific question on API if in suspension, viscosity, preservative, all excipient that could modify surface tension, viscosity, absorption

Do not forget that it is a combined product, points link with device: usability, leachable, leakage, ...

Q3 Extensive in vitro comparison => on the delivered product

PK study when possible

Clinical « equivalence » study

GUIDELINE QUALITY

Summary tables on tests required for development and for finished products QC

Quality data on Nasal product refers to inhaled products, in many cases

DIFFERENCIATION LOCAL VS SYSTEMIC EXAMPLE OF NASAL

The intention of administering an active substance into the nose could be to apply local treatment in the nose (such as e.g., products containing decongestants to be used in case of common cold or anti-inflammatory medication in case of allergic rhinitis).

Another common use of nasal administration is as an alternative to injections to achieve rapid systemic exposure to an active substance following absorption through the nasal mucosa. The approach to take when demonstrating **Therapeutic Equivalence (TE) will differ dependent on whether the product is intended for local or systemic treatment.**

CONCEPT PAPER NASAL THERAPEUTIC EQUIVALENCE/LOCAL

Currently, abridged applications for locally active substances are supported by in vitro data on TE, sometimes, but not always, complemented by pharmacokinetic or clinical data. A number of in vitro parameters are to be considered:

- Qualitative and quantitative composition
- Actuation volume, single actuation content, or mass of single dose
- Droplet size distribution
- Mass of droplets smaller than 10 μm
- Particle size distribution and morphological form of active substance for suspensions
- Spray pattern / plume geometry
- Rheological properties (e.g., thixotropy, viscosity)
- Surface tension
- pH
- Density
- Osmolality
- Buffer capacity

If TE cannot be concluded by means of in vitro data, in vivo data would be warranted unless the product is reformulated to fit the in vitro criteria.

IN VITRO TESTS

DROPLET AND PARTICLE SIZE CHARACTERIZATION

FOR LOCALLY APPLIED LOCALLY ACTING

Demonstrate equivalence of quantity / nature of drug available at site of action!

At least 3 batches of test and 3 batches of reference with at least 10 units per batch and stage (function of the tests)

Suspension: sedimentation, flocculation, re-homogenization and delivery device

Must compare delivery devices and their performance

CHARACTERIZATION COMPOSITION PHYSICO CHEMICAL

Similar qualitative/quantitative composition

Rheological characterization (e.g., thixotropy, viscosity),

Surface tension

Density

Ph

Osmolarity

Buffer capacity

Viscosity

CHARACTERIZATION LINKED WITH DEVICE

- Actuation volume, single actuation content, or mass of single dose
- Droplet size distribution
- Mass of droplets smaller than 10 μm
- Particle size distribution and morphological form of active substance for suspensions
- Spray pattern / plume geometry

For active substance(s) present in solid form, solubility, particle size, particle shape, density, rugosity, charge, polymorphic form and crystallinity => some must be done on delivered product to see the impact of the device

PARTICLE SIZE: MICROSCOPY, LASER, MDRS

On the delivered product

MDRS is a microscopy technique coupled with Raman determination spectrum. The technical characteristics of the MDRS equipment state the following:

- Raman can identify particles $\geq 1 \mu\text{m}$ by nature (API, Excipient)
- Microscopy can select particles $\geq 0.5 \mu\text{m}$ (but not its nature).
- However, only particles $>1 \mu\text{m}$ could be analyzed by Raman and “marked”. As a consequence, it could be then accurately measured only if $>1\mu\text{m}$.

ACCORDING TO APPARATUS CHARACTERISTICS

During the qualification of the equipment uncertainty of the measurement is of $0.4\mu\text{m}$ on particles of nominal diameter of 1.5, 4, and $8\mu\text{m}$ leading to more unprecise measurement on smaller particles up to $0.4/1.5 \times 100 = 27\%$ compared to $4\mu\text{m} = 10\%$ and $8\mu\text{m} = 5\%$.

The observed values of this parameter (bias) were of 10, 1 and 0.25% respectively.

PREPARATION

Only pseudo spherical particle could be accurately measured

Important to accurately set up

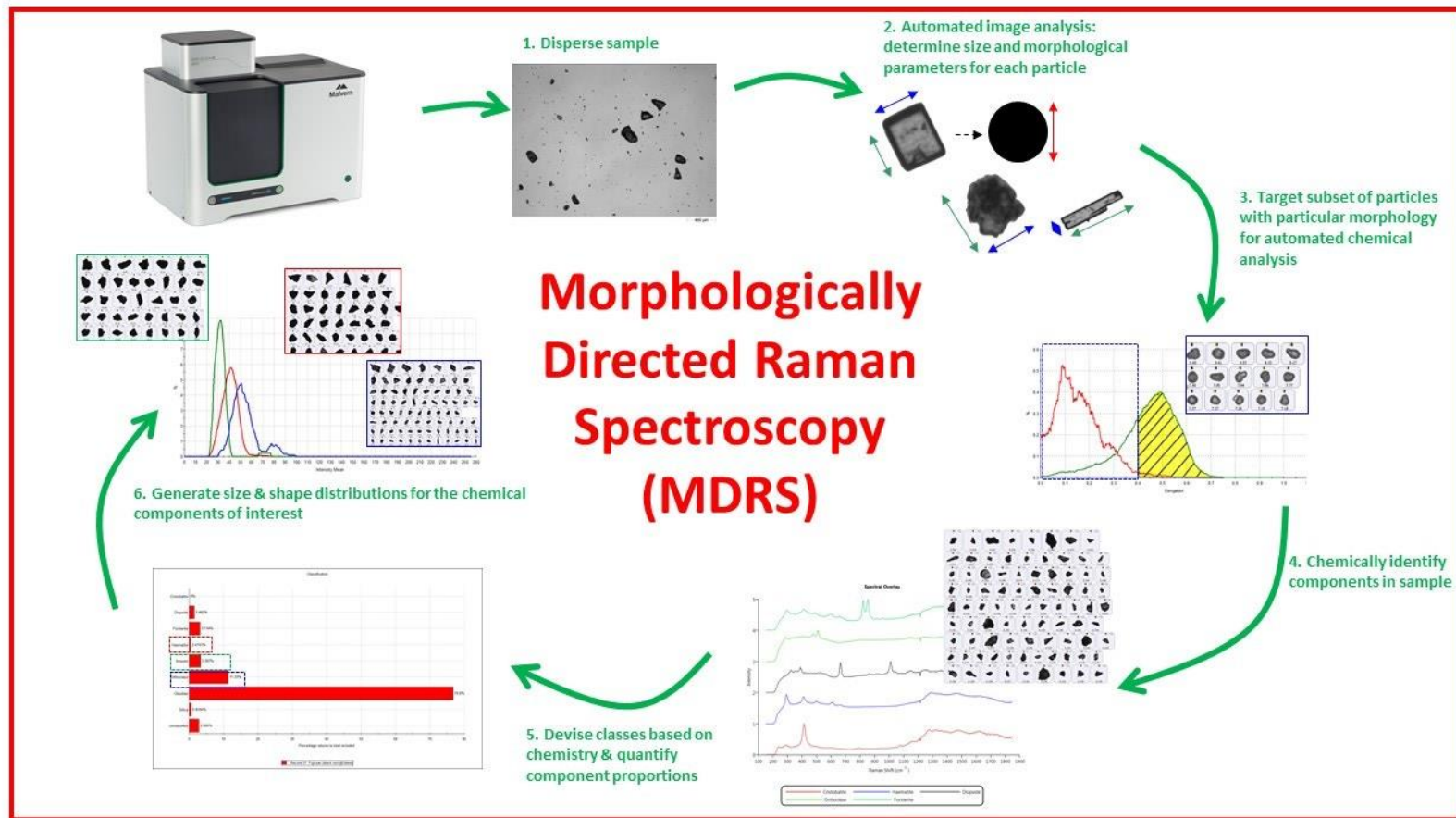
1. Sample preparation => plate

2. Particle imaging and morphology analysis: Small surface

3. Morphology filter selection

4. Particle Raman measurements and classification

5. Determination of minimum number of particles required and analyzed by run (multiple runs are often necessary)



<https://www.malvernpanalytical.com/en/products/technology/image-analysis/morphologically-directed-raman-spectroscopy>

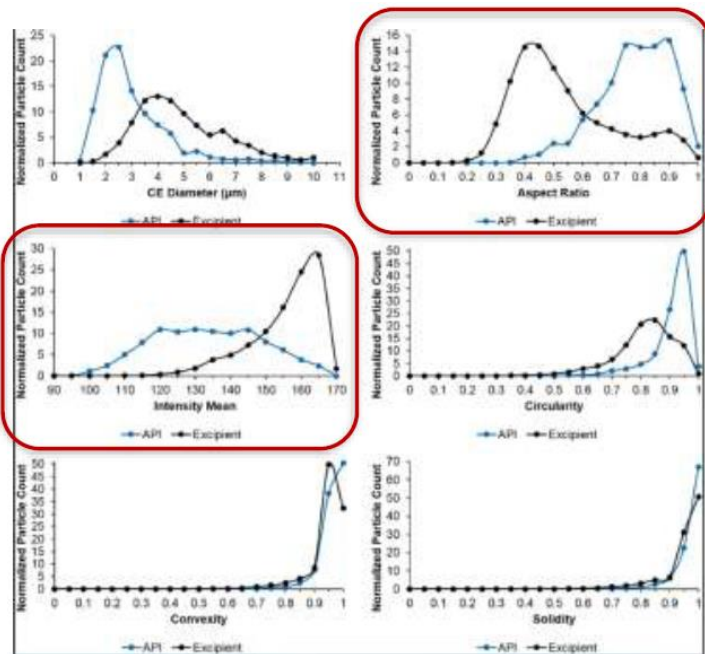
FILTERS / MUMBER OF PARTICLE

Aspect ratio roundness/ovality
Intensity / absence of aggregate

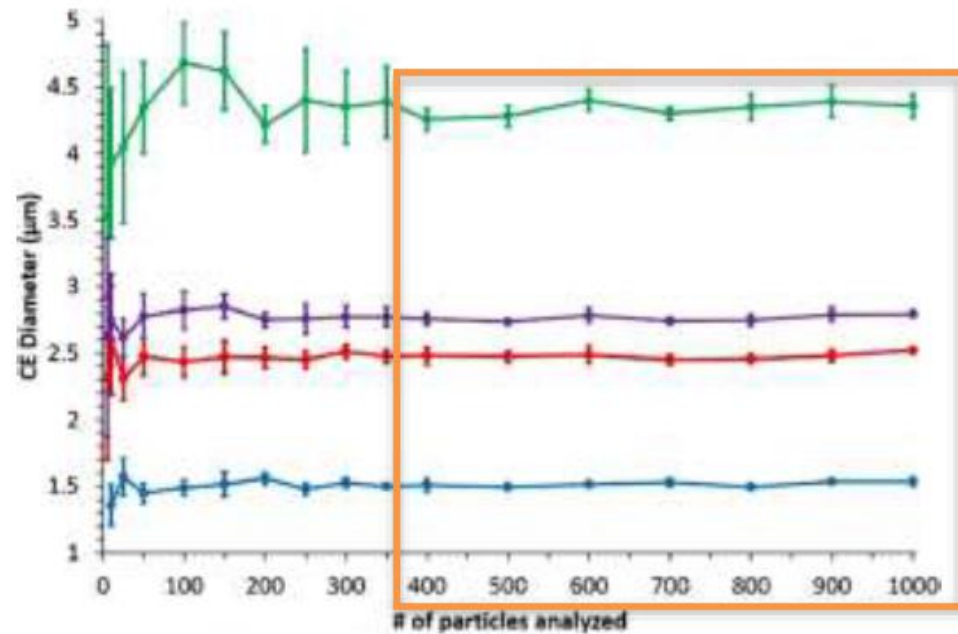
At least 400 to be counted

MDRS

Sample preparation Filter used



Number of particle



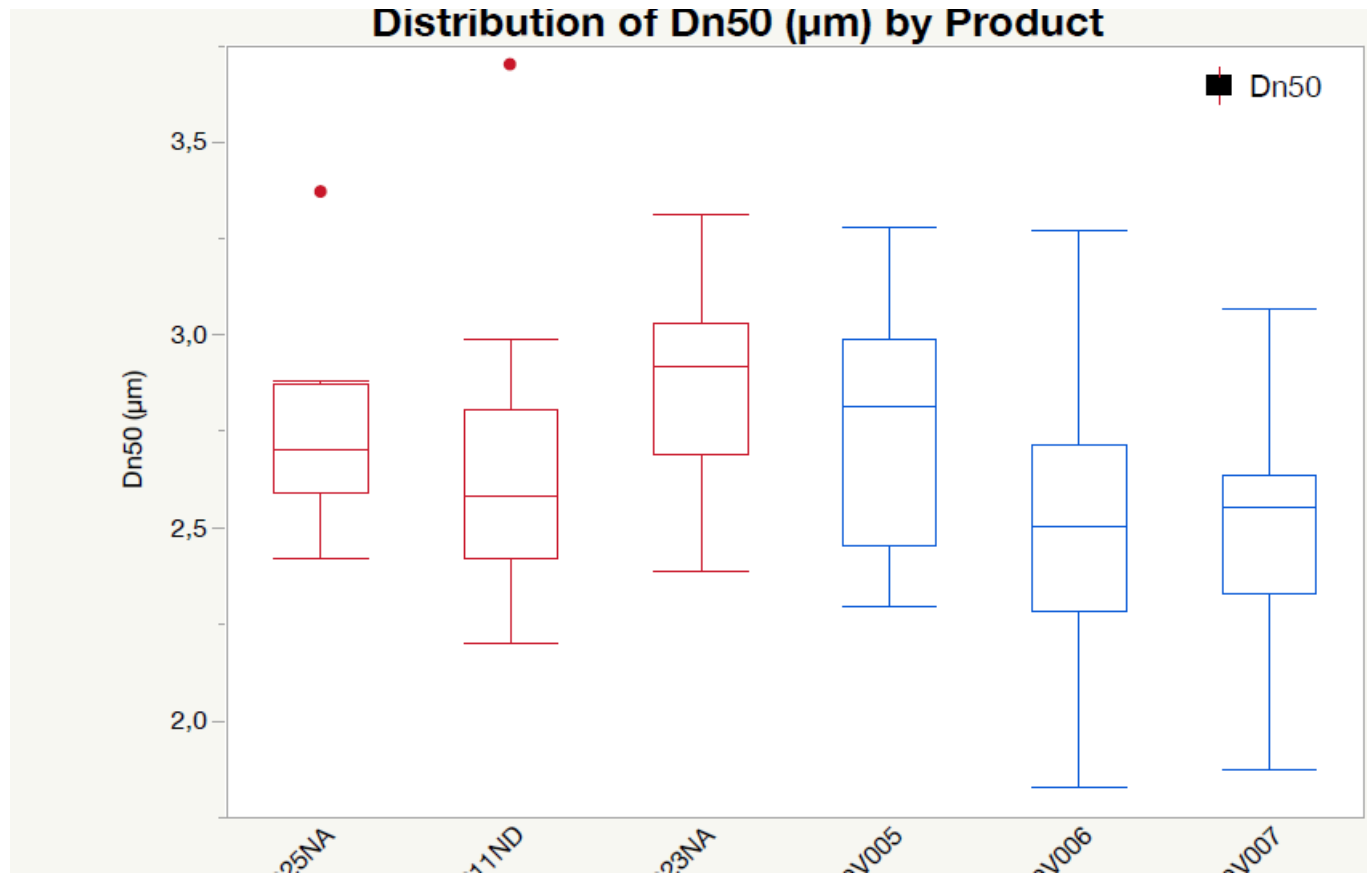
Thomas B J Analytical method development for characterizing ingredient-specific particle size distributions of nasal spray suspension products
J Pharm Sci 2021, 110, 2278-2788

PARAMETERS

- Diameter Diameter of a circle with the same area as the projected area of the particle images
- $D_n(0.1)$ Size of particle below which 10 % of the sample number lies
- $D_n(0.5)$ Size of particle below which 50 % of the sample number lies
- $D_n(0.9)$ Size of particle below which 90 % of the sample number lies
- $D_v(0.1)$ Size of particle below which 10 % of the sample volume lies
- $D_v(0.5)$ Size of particle below which 50 % of the sample volume lies
- $D_v(0.9)$ Size of particle below which 90 % of the sample volume lies
- Span $[D(0.9) - D(0.1)] / D(0.5)$

EXAMPLE OF RESULT

Unit Number	# Particles	CE Diameter (μm)			CE Diameter (μm)			Span	
		Dn(0.1)	Dn(0.5)	Dn(0.9)	Dv(0.1)	Dv(0.5)	Dv(0.9)	Number	Volume
1	746	1.58	2.61	3.72	2.40	3.37	5.66	0.82	0.97
2	565	1.90	2.77	3.91	2.49	3.48	4.58	0.73	0.60
3	452	1.79	2.72	3.68	2.38	3.25	4.37	0.69	0.62
4	501	1.53	2.73	3.98	2.49	3.47	5.04	0.90	0.74
5	108	1.65	2.56	3.31	2.31	2.94	3.62	0.65	0.45
6	446	1.99	2.87	3.91	2.51	3.44	4.85	0.67	0.68
7	707	1.51	2.79	3.74	2.55	3.26	4.64	0.80	0.64
8	291	2.07	2.98	3.96	2.63	3.48	4.52	0.63	0.54
9	104	2.06	3.03	4.12	2.66	3.45	4.82	0.68	0.63
10	731	1.59	2.71	3.73	2.42	3.28	4.46	0.79	0.62
Minimum	104	1.51	2.56	3.31	2.31	2.94	3.62	0.63	0.45
Maximum	746	2.07	3.03	4.12	2.66	3.48	5.66	0.90	0.97
Average		1.77	2.78	3.81	2.48	3.34	4.66	0.74	0.65
SD		0.22	0.15	0.22	0.11	0.17	0.52	0.09	0.14
%RSD		13%	5%	6%	4%	5%	11%	12%	21%



STATISTICAL ANALYSIS

Ln transformation

90 % CI

Acceptance limits

Role of Reference variability

REMARKS

For MDRS minimum number of particle should be justified as well as the filter selection (cut off) for both API and excipients, and duration of exposure for Raman spectra

DISSOLUTION FOR NASAL AND INHALATION PRODUCTS

Mentioned as research topics from early 80-90s

In the last decade many publications even from agencies for example (not limitative)

- 2017 Vincenzi (EMA) informed that dissolution was an ongoing discussion point in update of guideline (... but not kept)
- 2022 Boc and Newman (FDA) indicated that dissolution tests were able to differentiate formulations with different API particle size and referenced Hochhaus G, et al.
- Not introduced in current guidelines but accepted/requested by assessors

DISSOLUTION PARTICLE SIZE

Already linked between the two is made in ICH Q6A for various formulation and concerns.

... It is generally technically very difficult to measure polymorphic changes in drug products. A surrogate test (e.g., dissolution) (see Decision tree 4(3)) can generally be used to monitor product performance,

.... Quantitative acceptance criteria and a procedure for determination of particle size distribution may be appropriate for injectable suspensions. Developmental data should be considered when determining the need for either a dissolution procedure or a particle size distribution procedure

DISSOLUTION

Various apparatus were proposed and used.
For pure dissolution using Pharmacopeia apparatus, the most commonly cited are

USP 4 Flow Through Cell
Like injectable suspension

USP 2, USP 5 Paddle Over Disk
As described by Hochhaus

Both could respect sink conditions



FIRST PROBLEM SAMPLE

The sample introduced could be measure as per the mass (and not the dose) using the delivery system and assuming homogeneity

One or two actuations/puffs used.

The mass introduced could help to adjust the results to the mass as the precision of actuation could be of $\pm 15\%$ as mean or even to $\pm 25\%$ for some extreme values as per the EP

However, that suppose that mass is correlated to the dose ...

STUDY OF MASS DELIVERED VS DOSE DELIVERED RLD

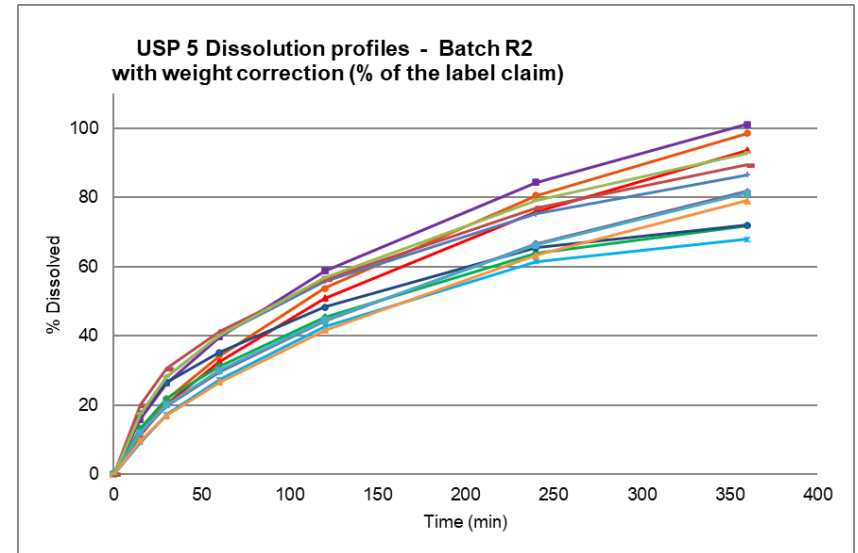
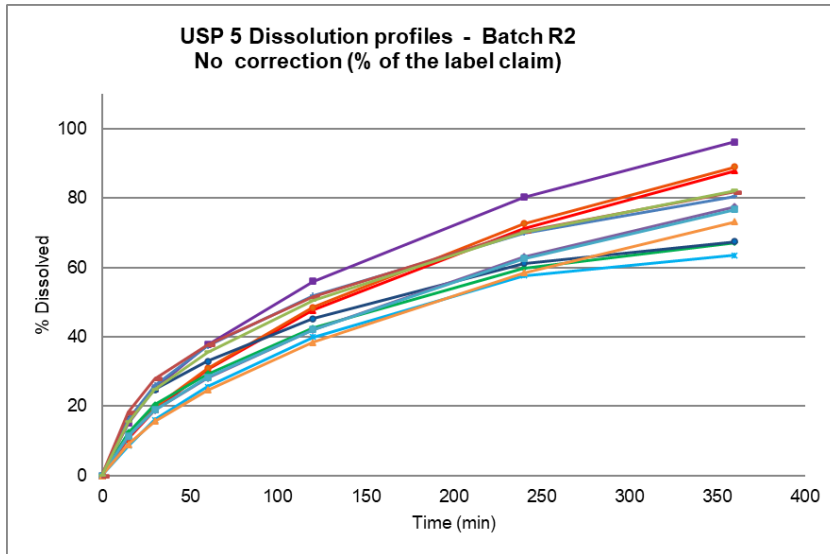
Using the test as per pharmacopeia the results of mass and dose delivered are as follow for drug CCCCC.

Type	Batch	N	SAC Dose μg		SAC Mass mg	
			Mean	Std	Mean	Std
Ref	X1	10	89.14	6.57	102.73	1.09
	X2	10	100.74	5.91	100.74	1.99
	X3	10	92.19	13.69	101.73	2.17

It could be observed that the mass delivered is correct for all formulations/drug but dose delivered is or not linked with the mass delivered, that lead to problems in the way to analyze/ normalize the results even if the results are within the acceptable limits of pharmacopeia for both.

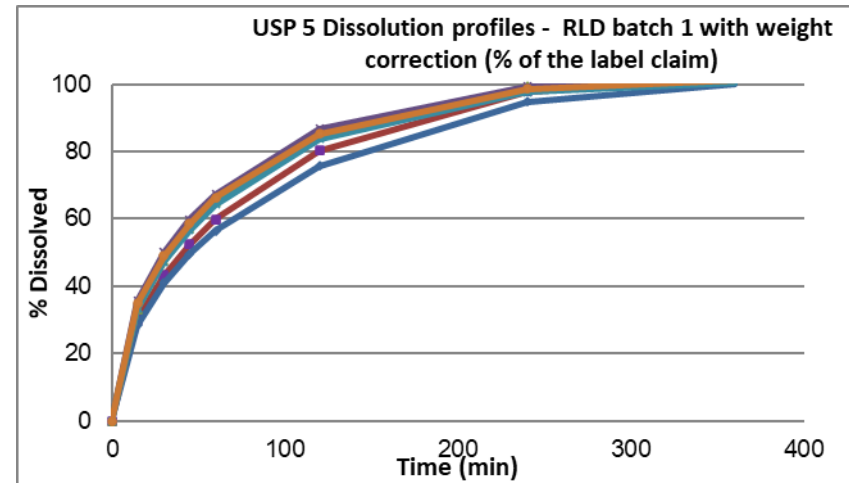
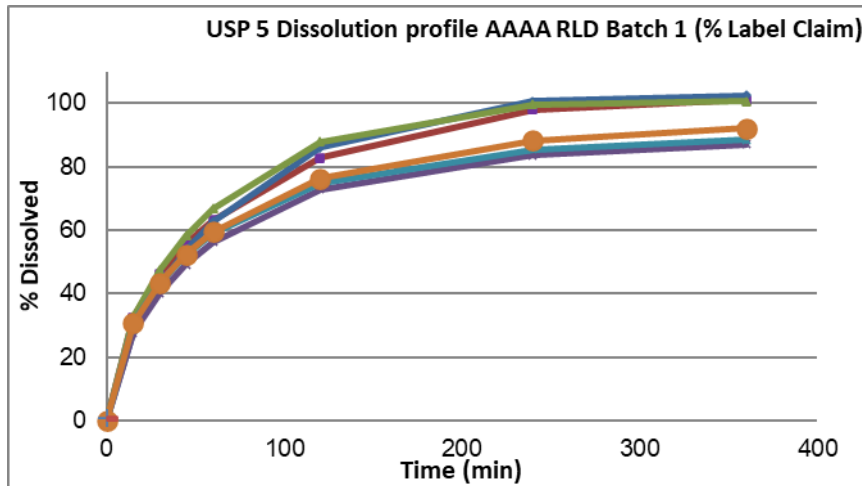
CORRECTION BY THE MASS DELIVERED DRUG CCCC

When mass and dose are not correlated



CORRECTION BY THE MASS DELIVERED DRUG BBBB

When mass delivered is connected to dose



However, mass does not mean dose ... and then the correction would not be of interest

Could correct by the final point => comparison of dissolution rate

CORRECTION ALL UP 100% (FINAL POINT)

Analyse sole rate

Assume that rate linked with particle morphology and solid state that is the basis of Noyes-Whitney equation

F2

The usual way to compare dissolution curves is F2.

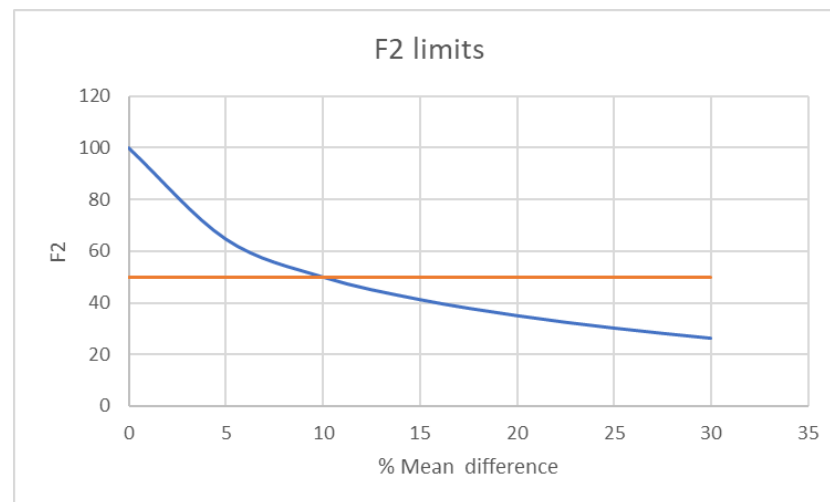
F2 is designed to detect a 10% difference between the test and the reference.

For solid oral dosage formulations, the dose must be with $\pm 5\%$

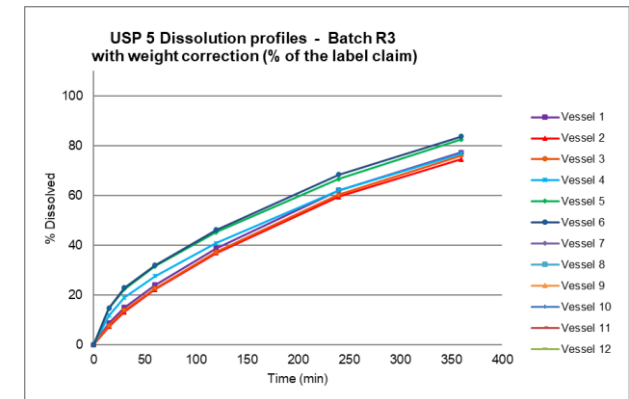
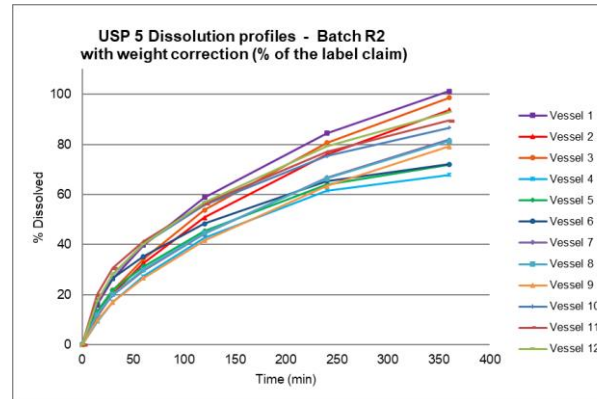
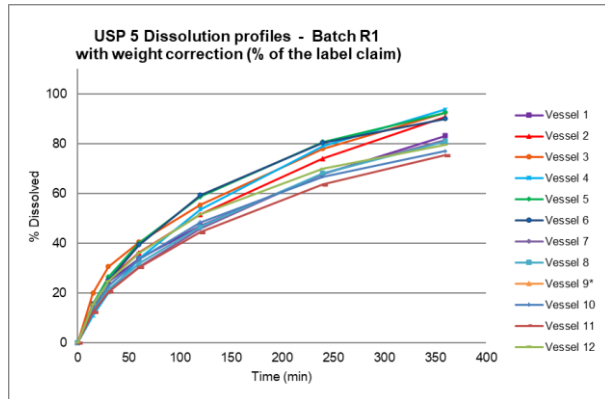
For Inhaled and nasal dosage formulation the dose must be with $\pm 15\%$

Is F2 limits at 50 adapted ?

Using 2 times the limits of dose results to 30% difference and $F2 = 26\%$



USP 5 VARIABILITY RLD BATCHES 1, 2, AND 3 DRUG BBBB



Depending of the batches, variability could be observed within and between batches

TEST STATISTICAL

EMA on Q1 Q2 similar composition, small variation accepted (see ICH M9/13B guideline to have idea)

EMA physico-chemical parameters Limits with $\pm 10\%$ limits

EMA Average Bioequivalence with $\pm 15\%$ limits for performance as based on cross reading with inhalation products. F2 for dissolution.

FDA Population based bioequivalence taking into account reference variability. PG is limited by US FDA at $\pm 10\%$ difference

Dissolution F2/bootstrap limits 10%

CONCLUSION

DISSOLUTION

No perfect solution ...

Dissolution represents solubilization of API and all parameters link to it: such as PSD, excipients, etc...

Do not forget that QC test could be requested ... dissolution more easy to implement in all labs vs MDRS?

Some MDRS sub contractors are located in UK outside of EMA formal region: cannot be used for QC testing ! ?

ACKNOWLEDGMENT

To P. SUPLIE and Substipharm for the fruitful discussions

REFERENCES

- Amini E, Kurumaddali A, Bhagwat S, Berger SM, Hochhaus G. Optimization of the Transwell® System for Assessing the Dissolution Behavior of Orally Inhaled Drug Products through In Vitro and In Silico Approaches. *Pharmaceutics*. 2021; 13(8):1109. Amini E, et al. Sensitivity of Pharmacokinetics to Differences in the Particle Size Distribution for Formulations of Locally Acting Mometasone Furoate Suspension-Based Nasal Sprays. *Mol Pharm*. 2023 Nov 6;20(11):5690-5700.
- Boc S. (FDA), Alternative BE Approaches and Considerations for Nasal Products SBIA 2022: Advancing Generic Drug Development: Translating Science to Approval
- Doub WH, Suman JM, Copley M, Goodey AP, Hosseini S, Mitchell JP. Laboratory Performance Testing of Aqueous Nasal Inhalation Products for Droplet/Particle Size Distribution: an Assessment from the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS). *AAPS PharmSciTech*. 2023 Oct 10;24(7):208.
- Eedara BB, Bastola R, Das SC. Dissolution and Absorption of Inhaled Drug Particles in the Lungs. *Pharmaceutics*. 2022 Nov 30;14(12):2667.
- Farias G, Shur J, Price R, Bielski E, Newman B. A Systematic Approach in the Development of the Morphologically-Directed Raman Spectroscopy Methodology for Characterizing Nasal Suspension Drug Products. *AAPS J*. 2021 May 18;23(4):73.
- Forbes B Richer N, Buttini F Chapter 10 Dissolution: A Critical Performance Characteristic of Inhaled Products? In *Pulmonary Drug Delivery: Advances and Challenges* Willey 2015
- Hochhaus G, et al. Evaluating Particle Size Differences of Suspension-Based Nasal Sprays Through In Vitro and Pharmacokinetic Approaches. *Respiratory Drug Delivery* 2022. Volume 1, 2022:47-54.
- Newman B. (FDA) , Nasal Products: Current Landscape and Recent Advancements SBIA 2022: Advancing Generic Drug Development: Translating Science to Approval
- Nokhodchi A, Chavan S, Ghafourian T. In Vitro Dissolution and Permeability Testing of Inhalation Products: Challenges and Advances. *Pharmaceutics*. 2023 Mar 18;15(3):983.
- Son Y, Horng M, Copley M, McConville Optimization of an In Vitro Dissolution Test Method for Inhalation Formulations May 2010 *Dissolution Technologies* 17(2)
- Vincenzi C. Update on the guideline on the pharmaceutical quality of inhalation and nasal products DDL2017 6-8 December 2017
Biobridges Sept 2026

?? ? ? ? ? ? ? ? ? ? ? ? ? ?

QUESTIONS

THANK YOU

E.mail: jean-michel.CARDOT@wanadoo.fr
