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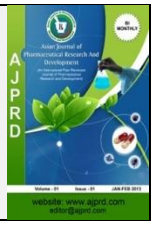

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Review Article

Nebulizer – In vitro Bioequivalence Testing Requirements: A review

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A B S T R A C T

Generic Nebulized aerosol is often perceived as inferior to their branded counterparts; however, they are safe and effective if they can meet the regulatory requirements. International regulatory agencies have developed recommendations and guidance for bioequivalence approaches of orally inhaled drug products (OIDPs) for local action. The objective of this article is to discuss the approaches can be used for to show bioequivalence requirement for Food and Drug Administration (FDA) in the United States of America (USA) with reference product. This review endorsed that inhalation solutions and suspensions undertaken to determine the drug substance delivery rate, total drug substance delivered and Aerodynamic assessment of nebulized aerosols [aerodynamic particle size distribution (APSD)/droplet size distribution (DSD)].These are the most important parameters in characterization, evaluation and bioequivalence studies of nebulizers. These devices operate continuously; once loaded and require little or no co-ordination on the part of user they proved that these devices suitable for weak, pediatric or geriatric patients. The regulatory agencies declared that; there is no specific requirements testing in case of nebulizers like Metered dose inhalers (MDI) and Dry powder inhalers (DPIs). In United States of America (USA) regulation falls under the auspices of centre for devices and radiological health (CDRH), and new devices require 510(k) premarket notification. This review outlines all the bioequivalence test parameter.

Keywords: *In-Vitro* equivalence, APSD, Unit Dose Content, delivered dose, Morphologically-Directed Raman Spectroscopy (MDRS)

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INTRODUCTION

In the development of generics, the aim is to duplicate the performance of an established product precisely so that both innovator and generic can be used interchangeably to deliver an identical therapeutic effect. Identifying *in vitro* analytical strategies and instrumentation that provide the required information to demonstrate BE helps developers avoid the costs and time associated with *in vivo* testing and extensive clinical trials .Inhalation solution and suspension delivers drug substance into the lungs by oral inhalation and include inhalation aerosols, inhalation sprays, inhalation solutions, inhalation suspensions, solution for inhalation and drug for inhalation solution dosage forms.[1]Conventional nebulizer having some limitations like sufficient supply of air to operate jet technology to atomizes the liquid, or they require electricity in case of ultrasonic nebulization and portability.[2]The most important characteristic of nebulizer performance is respirable dose provided for the patient. The respirable dose determined by the mass output of the nebulizer and size of droplets that are fabricated; the droplet size must 2-5 µm for airway deposition and 1-2 µm for parenchymal deposition[3] The regulatory guideline endorsed that delivered dose, aerodynamic particle size, particle size/droplet size are the primary characterization techniques for nebulizers including [4]

- Physical characterization of the formulation
- Minimum fill justification
- Shaking requirements
- Compatibility with diluents and/or co-administered drugs

The critical quality attributes to assess the *In-Vitro* equivalence between the generic and reference products are the dose delivery uniformity and particle size distribution profile.[5] The delivered dose should be similar to reference drug. The comparison of reference drug to generic form of drug in the case of particle size distribution tested by

multistage impactor and justified group of stages with suitable equivalence criteria.[6]

Bioequivalence testing parameter for nebulized Aerosol:

Generics are approved based on *In-Vitro* data where they are examined as a minor variation of the Reference product, since variations of the Reference product are sometimes accepted without the need of *In-Vivo* studies such as solutions for nebulization.

If the composition is qualitatively and quantitatively identical, approval can be conceded based on physicochemical similarity (e.g., viscosity, pH). If the composition is qualitatively or quantitatively different, more substantial in vitro testing is necessary and should be regulated using the nebulizer described in the labelling of the Reference product.

This *In-Vitro* comparison should include droplet size distribution, which should be guided taking into account the potential difference in hygroscopicity, and nebulizer efficiency, which may differ simply due to the presence of surfactants / preservatives.

Suspensions for nebulisations in addition to the requirements reported for solutions for nebulization, it is necessary to reveal closeness in crystallography and similar particle size distribution of the particles in suspension.[7] For establish bioequivalence with approved reference product FDA recommend population bioequivalence (PBE) statistical approach to determine equivalence. The detailed procedures for PBE analysis are published in FDA's product -specific bioequivalence recommendation for nebulized budesonide inhalation suspension. The key *In-Vitro* studies for BE assessment of nebulizer summarized below. Selection of testing parameter for in vitro bioequivalence is based on formulation requirement.

Polymorphic form & crystalline habit of the drug substance

Drug Substance can exist in different crystalline forms (polymorph), solvate/ hydrate forms (pseudo- polymorphs) and amorphous form as a result of the manufacturing and storage conditions. These different forms can have a profound effect on the quality or performance (e.g. Solubility, Bioavailability, efficacy and safety) of the drug product. For this reason, it is now regulatory requirement to conduct details analysis of polymorph of the drug substance.

The identification of different (pseudo) polymorphs of an active pharmaceutical ingredient in formulation is essential for inhalation suspension formulation. Polymorph present in generic formulation similar with reference drug product.

Resemblance of polymorphic form of the drug substance can be confirmed by X-ray diffraction. The use of synchrotron XRPD enables the detection and identification of different polymorphic forms of in formulation with low concentrations of drug substance[8] .For efficient deposition in the lungs, physical properties of the aerosolized drugs are critical: the particles should have an ideal size and morphology that provide optimal aerodynamic performance[9] The aerodynamic properties of the drug particles, their binding to the excipient and their stability all impact upon the efficacy of dosing. Therefore, the crystal size, habit, shape and surface of micronized material need to be fully supervised in order to optimized the aerodynamic properties. Particle visualization by optical microscope offers easy, efficient and reliable way to get information about the crystal size, habit, shape, particle size distribution. [10]

Unit Dose Content (UDC) of drug in the ampules.

Weight of content formulation, concentration of drug substance (mg/mL) and drug substance content (mg) in each vial should be evaluate in test drug product. Inhalation suspension packaged in glass vial or plastic ampule Unit Dose Content (UDC) should be evaluated by content uniformity.

For inhalation solution packaged in glass vial or plastic ampule Unit Dose Content (UDC) can be evaluated by weight variation of individual vial or ampule for solution base formulation and suspension base formulation content of or vial ampule by validated analytical method. For multiple strength drug product comparative Unit Dose Content (UDC) of need to established in lower strengths of the test and reference products.

Suitable statically measure should be used for establishment of equivalency with reference drug product[11] In general, at least 10 vials from single batch required to establish comparative unit dose content (UDC) the test and reference products.

Comparative Mean Nebulization Time (MNT) and Mean Delivered Dose (MDD).

These tests are performed to assess the rate of delivery to the patient and the total drug substance delivered to a patient using standardized conditions of volumetric flow rate. Breath simulators empower the investigation of the impact of breathing profiles on drug delivery performance, and their use helps clarify the different clinical efficacy of nebulized formulation in different patient groups.

Nebulisers operates steadily once loaded and activated. It determines the inhalation rate of the drug and finally the amount of active delivered to the patient.[12][13]Delivered dose testing is carried out to determine the total amount of drug that the patient might be anticipated to receive during a treatment period.[14] Two distinct metrics are delineated and dignified: the active substance delivery rate and the total active substance delivered.

Reflecting the mode of operation of nebulisers, delivered dose testing is conveyed using well defined breathing profiles for specific patient types. The defined profiles for child, infant and neonate patients are based on significantly smaller volumes, higher breathing frequencies and different inhalation/exhalation ratios.

The delivered dose can be measure by Copley breathing simulator as shown in figure 1 and test should be conduct as per USP<1601>.The dose delivered from the nebulizer on an inspiratory filter during a breath simulation experiment generally performed at standard adult breathing pattern (500 mL tidal volume, I: E 1:1, 15bmp). Delivered dose collected on the inspiratory filter during breathing simulation should be comparable between Test drug product and reference drug product.[15, 16, 17]

Figure 1: Deliver Dose testing set up of nebulizer [18]

It is recommended that according to new monograph breath simulator for delivered dose testing reflecting the routine operation of nebulizer and commercially available breathing simulator that is able to generate the breathing profiles specified in Table 1is used for the test. The breathing profile indicated for adults is used unless the medicinal product is specifically intended for use in paediatrics, when alternate patterns should be used, as indicated in Table 1. The length of the time interval ensures that sufficient drug deposited on inhalation filter for quantitative analysis. A time of 60 ± 1 sec must allow sufficient drug substance deposition on the inhalation filter to allow quantitative analysis. If the filter is soaked the product, this time can be decreased. at the end stops the nebulizer. The total mass of drug substance delivered by summing the mass of drug substance collected on all inhalation filters. Mean Nebulisation Time (MNT) and Mean Delivered Dose (MDD) characterized at the labeled flow rate and ensure that mist is no longer coming out from the mouthpiece of respective nebulizer device.[19]

Table 1: Breathing simulator specification[20]

Parameters	Specification			
	Adult	**Neonate**	**Infant**	**Child**
Tidal volume	500 mL	25 mL	50 mL	155 mL
Frequency	15 cycle/min	40 cycle/min	30 cycle/min	25 cycle/min
waveform	Sinusoidal	Sinusoidal	Sinusoidal	Sinusoidal
Inhalation: exhalation ratio	1:1	1:3	1:3	1:2

Drug substance particlesize and agglomerate.

The main purpose of particle size characterization in the pharmaceutical industry is to collect quantitative data on mean particle size, particle size distribution, and particle shape. The other purpose is to ensure the quality of the finished drug product. Particle size characterization can be done using various commercially available instruments. Different instruments are based on different techniques, sometimes even a combination of techniques. Comparative drug particle morphology and agglomerate and particle size distribution (PSD) due to process specially in suspension formulation (in the ampoule) shall be evaluate in test drug product and it should be comparable with reference drug product. Drug particle morphology and agglomerate and particle size distribution (PSD) should be evaluated by validated analytical method. Validation should establish method sensitivity to identify the drug particle size over the expected size range in the suspension. Sameness of particle size of drug and agglomerates in the nebulized aerosol samples were collected in a New Generation Cascade Impaction, then samples were combined to run through a Coulter Counter or also considered supportive data generated by a novel in vitro method. Morphologically-Directed Raman Spectroscopy (MDRS) or Morphology G3 with Rama spectroscopy as shown in figure 2, can use to characterize the particle size distribution (PSD) of active pharmaceutical ingredient (API) in the drug product. One major limitation of the MDRS method is its inability to measure particle size below 1µm. Hence, while applying MDRS method, users may need to use orthogonal methods to assess the submicron API particles. [21-22] With the help Morphologically-Directed Raman Spectroscopy (MDRS), possible to determine whether the size and shape of an API has been altered during processing.

Figure 2: Morphologically-Directed Raman Spectroscopy for API particle Size and agglomerates[23]

A notable application has recently highlighted by FDA, which specifically referred to MDRS in information it released about its approval of an abbreviated new drug application (ANDA) for a generic version of Nasonex (mometasone), a locally-acting suspension-based nasal spray for the treatment of allergies. Demonstrating the bioequivalence (BE) of a locally-acting product is challenging because traditional pharmacokinetic approaches based on measuring the level of drug in the bloodstream are not valid. FDA accepted *in-vitro* data measured using MDRS instead of clinical endpoint study, with the technique enabling measurement and comparison of the particle size of the drug particle within the formulation both before and after delivery. Here, MDRS proved key to the success of the submission, setting a precedent for other generics developers to avoid the expense and time associated with a clinical BE determination via analogous analyses. [24]

Aerodynamic particle size distribution.

Characterizing the in vitro particle size distribution of inhalation products using cascade impaction has become a standard approach to describe the aerodynamic particle size distribution (APSD) performance of these products. The aerodynamic diameter of an aerosol particle is equal to the diameter of a sphere of unit density whose gravimetric settling velocity is same. The aerodynamic size distribution defines the manner in which an aerosol deposits during inhalation. Nebulized products need to be size-characterized at flow rates lower than the range that is typically used for powder inhalers. The CEN standard recommends a flow rate of 15 L/min because this value represents a good approximation to the mid-inhalation flow rate achievable by a healthy adult breathing at 500mL tidal volume.

When the flow rate at 15 L/min neither pre separator works nor MOC (micro orifice collector). The pre separator dominated by gravitational forces loses its effectiveness in the flow rate of 15 L /min (generally used as a replacement for paper back up filter) because the predicted cut off diameter significantly increased. At this flow rate MOC may not work efficiently; it could not collect ultra-fine particles below stage 7 cut off diameter.[25-26]

Although low-angle laser light-scattering instruments (laser diffractometers) can provide rapid size-distribution measurements of nebulizer-generated aerosols, these techniques do not detect the drug substance and metered-dose inhalers the aerodynamic droplet size distributions nebulized of aerosols can determined by NGI as shown in figure 3 in accordance with U.S. Pharmacopoeia (USP) <1601>. The NGI body and components, including the cups, micro orifice collection filter, and induction port, need to set up and cool to 5°C. The use of the NGI cooler is also a more efficient process than using the fridge to cool the NGI.[27]

The characteristics of the aerosol can be assessed using several parameters including fine particle mass of drug collected in individual stages, fraction (FPF), MMAD, and fine particle dose (FPD). The MMAD is the most significant parameter defining particle size distribution, i.e. drug deposition. Theoretically, a mono disperse aerosol will exhibit a GSD of 1.0, in practice however, a GSD of ≤1.22 is considered as mono disperse particle size distribution. [28]

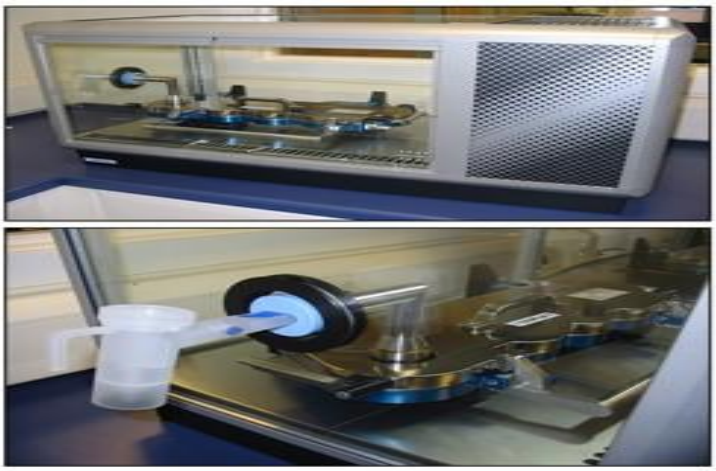

Figure 3: APSD testing Set up of nebulizer[29]

Aqueous droplet size distribution of the nebulized aerosol by a Laser diffraction method.

Laser diffraction-based particle sizing is finding increased application in the area of nebuliser characterization the technique allows for the real-time measurement of the size of droplets produced during nebuliser operation.[30-31]The aerosol particles are passed through the infrared laser causing refraction and diffraction of light from the particle surface. The angle of the light scattering depends on the size of the particle. A small particle will diffract light at a large angle and a large particle diffracts light at a small angle. The diffracted light passes through a lens and is detected on a plate positioned at the lens focal length. This plate consists of concentric rings and the detection of diffracted light by these rings determines the size of the particle that diffracted the light. A vacuum arranged opposite the nebulizer entrained the aerosol through the laser beam and prevented any particles re-entering the beam path.[32]An average droplet size distribution shall be calculate based on five 5-second measurements with an interval of 10 seconds. Spray tech as shown in figure 4 with inhalation cell are used to determine the aqueous droplet size distribution of the nebulized aerosol by a Laser diffraction method.

Figure 4: Spray tech for aqueous droplet size distribution testing [33]

CONCLUSION

Nebulisers, unlike other orally inhaled products (OIPs), do not require the coordination of inhalation with device actuation, making them especially suitable for those with poor coordination or lung function, such as geriatric or paediatric patients. They are also useful for delivering high drug doses over long periods of time. The regulatory frame work and pharmacopoeia monographs for nebulisers have changed considerably over the last decade. All OIPs now share a harmonised approach to test base on the characterisation of a specific formulation/ device. Furthermore, the tests specified for nebuliser testing now robustly scope their performance for specific patient groups the specific requirements for dose delivery testing and aerodynamic particle size distribution (APSD) measurement for this important class of OIPs. For nebulized products, the APSD tests are to be conducted per the RLD labelling and using the same nebulizer(s) as specified in the RLD label. FDA employs a statistical method, PBE, for equivalence analysis on majority of in vitro BE tests. The sponsors are encouraged to submit data on individual stages, as well as key parameters describing particle sizing such as ISM, MMAD, GSD, and FPM. The deployment of *in vitro* methods for demonstrating bioequivalence will tremendously expedite the development and reduce the cost for developing generic locally-acting drug products. It signifies a major advancement in the US regulatory science and has significant impact on other complex drugs.

REFERENCES

1. Byron Pr. United States Pharmacopeia recommendations for the testing of inhalers. Journal of aerosol medicine. 1998 ;11:S-11.
2. Faarc Dr. Nebulizers: principles and performance. Respiratory care. 2000; 45(6):609.
3. Newman SP. Nebuliser therapy: scientific and technical aspects. Draco; 1989.
4. Dennis J, Berg E, Sandell D, Kreher C, Karlssin M, Lamb P. European Pharmaceutical Aerosol Group (EPAG) nebulizer sub-team: assessment of proposed European Pharmacopoeial (Ph. Eur.) monograph Preparations for Nebulization. J Aerosol Med. 2007; 20: 40.
5. Hess D. Aerosol Delivery Devices in the Treatment of Asthma; Respiratory Care, 2008; 53(6):699-725.
6. Hou S, Wu J, Li X, Shu H. Practical, regulatory and clinical considerations for development of inhalation drug products. Asian journal of pharmaceutical sciences. 2015; 10(6):490-500.
7. Lee, Sau L., Bhawana Saluja, Alfredo García-Arieta, Gustavo Mendes Lima Santos, Ying Li, Sarah Lu, Shuguang Hou et al. "Regulatory considerations for approval of generic inhalation drug products in the US, EU, Brazil, China, and India." 2015:1285-1304.
8. Egusa, Kenji, Fumiaki Okazaki, Joerg Schiewe, Ulrike Werthmann, and Markus Wolkenhauer. "Identification of Polymorphic Forms of Active Pharmaceutical Ingredient in Low-Concentration Dry Powder Formulations by Synchrotron X-Ray Powder Diffraction." Drugs in R&D 17, 2017; (3):413-418.
9. Patton, J. S., & Byron, P. R. Inhaling medicines: delivering drugs to the body through the lungs. Nature Reviews Drug Discovery, 2007; 6(1):67-74.
10. Selby, Matthew D., Pieter D. de Koning, and Dannielle F. Roberts. "A perspective on synthetic and solid-form enablement of inhalation candidates." Future medicinal chemistry. 201; (13):1679-1701.
11. https://www.accessdata.fda.gov/drugsatfda_docs/psg/Budesonide_Inhalation_Sus_2092
12. Berlinski A, Hayden JB. Optimization of a procedure used to measure aerosol characteristics of nebulized solutions using a cooled next generation impactor. Journal of aerosol medicine and pulmonary drug delivery. 2010; 23(6):397-404.
13. Dennis J, Berg E, Sandell D, Ali A, Lamb P, Tservistas M, Karlsson M, Mitchell J. Cooling the NGI-an approach to size a nebulised aerosol more accurately. Pharm Europa scientific notes. 2008; (1):27-30.
14. Hiller C, Mazumdar M, Wilson D, Bone R. Aerodynamic size distribution of metered-dose bronchodilator aerosols. American Review of Respiratory Disease. 1978; 118(2):311-7.
15. Peters TM, Chein H, Lundgren DA, Keady PB. Comparison and combination of aerosol size distributions measured with a low-pressure impactor, differential mobility particle sizer, electrical aerosol analyser, and aerodynamic particle sizer. Aerosol Science and Technology. 1993; 19(3):396-405.
16. Fromen CA, Shen TW, Larus AE, Mack P, Maynor BW, Luft JC, DeSimone JM. Synthesis and characterization of monodisperse uniformly shaped respirable aerosols. AIChE Journal. 2013; 59(9):3184-94.
17. Marple VA, Olson BA, Santhanakrishan K, Roberts DL, Mitchell JP, and Hudson-Curtis BL: Next generation pharmaceutical impactor: a new impactor for pharmaceutical inhaler testing. Part III. Extension of archival calibration to 15 L/min. J Aerosol Med. 2004; (17):335–343.
18. http://dissolutiontech.com/issues/201802/inews-copley02.php
19. Copley M. Improving inhaled product testing: methods for obtaining better in vitro-in vivo relationships. Pharmaceutical Technology. 2013; 2(2):37.
20. https://www.copleyscientific.com/files/ww/news/.
21. Scientific Considerations for the Review and Approval of First Generic Mometasone Furoate Nasal Suspension Spray in the United States from the Bioequivalence Perspective, AAPS J. 2019; 7;21(2):14
22. https://www.accessdata.fda.gov/drugsatfda_docs/anda/2009.
23. https://www.malvernpanalytical.com/en/products/product-range/morphologi-range/morphologi-g3-id
24. http://www.pharmtech.com/morphologically-directed-raman-spectroscopy-uncovering-good-bad-and-ugly-drug-formulations.Song X, Hu J, Zhan S, Zhang R, Tan W. Effects of temperature and humidity on laser diffraction measurements to jet nebulizer and comparison with NGI. AAPS Pharm SciTech. 2016; 17(2):380-8.
25. USP<1601>Page no. 7875, 2018, USP 41.
26. JayeshKumar, S.M., Yashvi, P., Bhimani, B., Patel, G., Patel, J. and Patel, U. Nebulization Therapy for Lung Cancer. *Research Journal of Pharmacy and Technology*, 2019; 12(2):920-934.
27. Mitchell JP, Nagel MW, Nichols S, Nerbrink O. Laser diffractometry as a technique for the rapid assessment of aerosol particle size from inhalers. Journal of aerosol medicine. 2006; 19(4):409-33.
28. https://www.mspcorp.com/pharmaceutical/ngi-cooler.
29. Vecellio None L, Grimbert D, Becquemin MH, Boissinot E, Le Pape A, Lemarié E, Diot P. Validation of laser diffraction method as a substitute for cascade impaction in the European Project for a Nebulizer Standard. Journal of aerosol medicine. 2001; 14(1):107-14.
30. Bouwman AM, Heijstra MP, Schaefer NC, Duiverman EJ, Lesouëf PN, Devadason SG. Improved efficiency of budesonide nebulization using surface-active agents. Drug delivery. 2006; 13(5):357-63.
31. Pham S, Ferguson GT, Kerwin E, Goodin T, Wheeler A, Bauer A. In vitro characterization of the eFlow closed system nebulizer with glycopyrrolate inhalation solution. Journal of aerosol medicine and pulmonary drug delivery. 2018 ;31(3):162-9.
32. https://www.malvernpanalytical.com/en/products/product-range/spraytec.