In vitro bioequivalence testing of nasal sprays

Interest in new test methods for bioequivalence of inhaled and nasal drug products is growing—are there alternative ways forward?

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Bioequivalence testing compares the performance of two different products: an innovator and a generic nasal spray, for example, or perhaps two different versions of a nasal spray during ongoing product development. As evidenced by a two-day workshop on demonstrating bioequivalence of locally acting inhaled drugs scheduled for March 9-10, interest in bioequivalence testing for orally inhaled and nasal drug products (OINDPs) has soared recently.

Sessions on this topic at the 2008 AAPS annual meeting in November were well attended and sparked a great deal of discussion. The March workshop, sponsored by the Product Quality Research Institute (PQRI) and the Inhalation and Nasal Technology Focus Group (INTFG) of the American Association of Pharmaceutical Scientists (AAPS), will feature approximately 15 presentations by industry, academic, and regulatory experts.

A reliance on *in vitro* testing could aid in the timely approval of generics and could reduce time and expense for the development of novel products. Cost effective and efficient methods to establish bioequivalence would benefit the development of both brand-name and generic pharmaceuticals, saving both companies and consumers significant amounts of money. Much of the current interest in bioequivalence stems from a desire to introduce improved methods of testing to achieve these objectives, and several new, potentially more meaningful, *in vitro* methods show promise in that area.

The need for more meaningful tests

Bioequivalence for drugs that reach their cellular targets via the bloodstream is typically established by demonstrating achievement of an indistinguishable rate and extent of absorption into plasma. However, concentrations of locally-acting drugs delivered by nasal sprays and inhalers often fall below detectable limits in the plasma. Furthermore, the resulting plasma concentration likely has little relevance to these drugs' biological effects [1]. Therefore, the pharmaceutical industry and regulators have a need for *in vitro* tests to establish bioequivalence of these drug products in lieu of pharmacokinetic, pharmacodynamic, or clinical data.

However, *in vitro* tests have proven more sensitive to differences in nasal and pulmonary drug delivery than have clinical data [2], potentially causing overvaluation of sensitivity and leading to findings of significant, but perhaps clinically irrelevant, differences between the test and the reference. The 2003 FDA Draft Guidance "Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action" recommends seven tests for nasal sprays, most of which characterize various physical aspects of the aerosolized spray (Table 1) [3].

While these recommended tests discriminate among nasal sprays actuated into the air, none of them teaches us much about the specific sites of droplet deposition within the nasal cavity. In addition, methods can be designed to show no differences between products under one set of experimental conditions and differences under other protocols.

To what extent can we justify using complex and expensive equipment such as a laser light sheet or high speed camera to determine the spray pattern and plume geometry when these tests have no proven relationship to deposition of a product sprayed within the confines of the nose? Does the sensitivity of currently practiced *in vitro* tests result from robotic actuation that fails to represent realistic variations in actuation by a cohort of patients? An exploration of less burdensome, less expensive, yet potentially more meaningful *in vitro* tests that teach us about the performance of products in the hands—or perhaps more correctly, the noses and lungs—of patients follows.

Actuation of nasal sprays during *in vitro* tests

Replacing a rigid dip tube with a weighted, flexible tube for tests of spray weight and tail-off characteristics demonstrates that *in vitro* tests performed in the vertical position may not be clinically relevant when the instructions direct the patient to spray the bottle at an angle. Bottle orientation during pump actuation affects the priming, re-priming, and tail-off profile of a product. Non-vertical positions may also result in partial sprays because the tube may or may not be fully submerged in the formulation when the bottle is held at an angle. Since most nasal spray bottles are opaque, the patient likely will not know in which direction the dip tube bends.

While *in vitro* nasal spray studies often use automated actuation stations that hold the pump in the vertical position to spray the products, the package inserts included with different brands of nasal sprays recommend various pump positions, meaning that a vertical orientation may not accurately represent actuation by patients. Although the package insert for Glaxo's Flonase prompts patients to spray vertically with the head tilted forward, others such as Aventis's DDAVP direct the patient to spray at an angle.

Testing multiple nasal spray units at an angle without pre-selecting a specific dip-tube orientation probably best represents the way most patients use nasal spray products. Such a practice undoubtedly results in more variable *in vitro* data compared to a vertical bottle orientation, particularly as the product approaches its label claim number of sprays, but it provides a more representative indication of the nature of the spray that enters the patient's nose.

A study involving actuation of 5 over-the-counter nasal sprays with the bottles at a 30° angle to the vertical found that dip tube orientation affected both the number of full sprays and the tail-off profile. Weighted, flexible dip tubes whose tips always position themselves at the lowest point in the bottle regardless of orientation significantly increased the number of full sprays in four out of five products when compared to inherently curved, rigid dip tubes actuated in their worst-case orientation. The flexible dip tubes also reduced the duration of the tail-off period in 3 out of 5 products (Table 2).

Table 1

***In vitro* tests recommended by FDA for determining bioequivalence in nasal sprays**

Test	Technique
Single actuation content through container life	Dosage unit sampling apparatus
Droplet size distribution	Laser diffraction
Drug in small particles/droplets	Cascade impaction
Drug particle size distribution (only for suspension nasal sprays)	Microscopy
Spray pattern	TLC plate impaction or laser light sheet
Plume geometry	Flash photography, high-speed video, or laser light sheet
Priming and repriming	Dosage unit sampling apparatus, established from beginning lifestage data obtained in single actuation content study

Table 2

Average number of full sprays and sprays in the tail-off period achieved using rigid and flexible dip tubes (standard deviation in parentheses)

	Full Sprays		Tail-off period	
Nasal Spray Product	Rigid	Flexible	Rigid	Flexible
Afrin	69.0 (4.2)	101.8 (12.4)	11.3 (4.9)	12.3 (8.7)
Ayr	106.4 (3.3)	134.2 (1.9)	17.3 (2.1)	6.3 (1.2)
Nostrilla	103.6 (6.1)	134.8 (4.6)	25.0 (7.0)	10.0 (1.0)
Rite Aid	220.8 (3.8)	293.9 (2.2)	57.3 (16.0)	9.0 (1.7)
Zicam	83.2 (3.4)	87.0 (8.8)	19.0 (2.0)	5.3 (2.3)

Patient-relevant settings for *in vitro* tests

The FDA recommends using automatic actuation systems with settings representative of the patient population for *in vitro* bioequivalence testing to reduce variability introduced by analyst hand spraying [3]. Pharmaceutical companies need only to report and justify one set of settings used with an automatic actuation station. However, utilizing a range of settings to show that *in vitro* performance remains consistent would provide more realistic and clinically useful data.

The patient's hand technique in actuating the nasal spray affects product performance because different actuation velocities or forces affect the spray weight, droplet size distribution, and spray pattern [4-7], possibly contributing to differences in deposition in the nasal cavity.

A study to determine patient relevant settings for controlled automatic actuation systems used to evaluate *in vitro* Flonase performance with 20 adult participants produced unique and highly variable sets of force and displacement profiles, suggesting that testing the product using a range of actuator settings would prove beneficial. [Fig. 1].

In vitro tests for suspension nasal spray products

One possible method of establishing suspension product uptake equivalence involves the absorption and metabolism of a nasal suspension by human airway epithelial cells grown in the laboratory. The FDA has differing requirements for bioequivalence testing for nasal suspensions than for nasal solutions based on the assumption that drug particle size distribution (PSD) in suspension formulations may affect the rate and extent of drug availability to nasal sites of action and to the systemic circulation. However, a study of micronized beclomethasone dipropionate (BDP) suspension using the Calu-3 cell line as a model found that the primary drug particle size does not necessarily influence the amount of drug uptake by the cells.

BDP uptake by Calu-3 cell monolayers, as analyzed by LC-MS, demonstrates that solutions exhibit a linear increase in uptake with increasing BDP concentration, but suspensions with differing drug sizes show no significant difference. BDP uptake may occur via passive diffusion, and the concentration of 17-BMP, the major metabolite, depends on that of the starting dissolved BDP concentration. The study shows that uptake of BDP by Calu-3 cells incubated with BDP drug solution (0.2-24 µM) for 2 hours depends on concentration (Fig. 2a). As expected, the concentration of the major metabolite, 17-BMP,

Figure 1

Four examples of displacement and force versus time profiles generated by hand

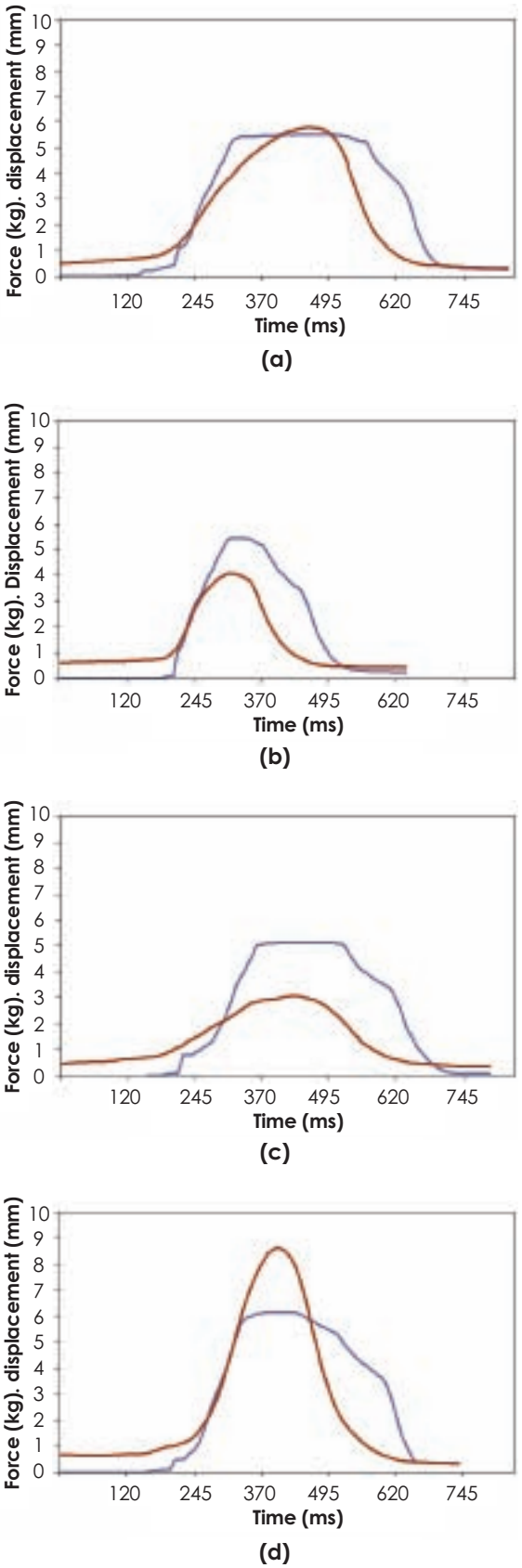
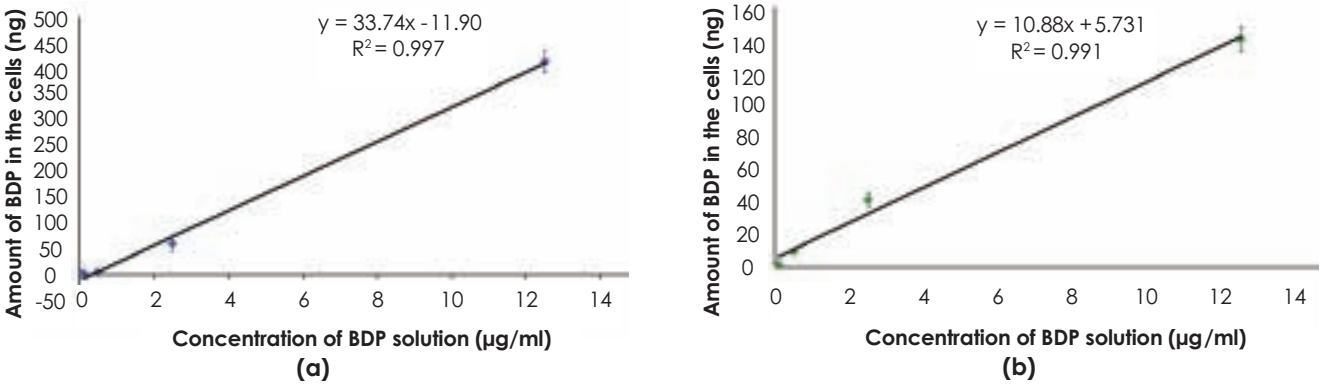


Figure 2

The percentages of BDP internalized (left) and metabolized (right) by Calu-3 monolayers incubated with BDP drug solutions



increases linearly with that of the added BDP solution (Fig. 2b).

For suspensions, however, no significant difference appears in cells incubated with two BDP suspension formulations with drug particle volume median diameters of 2.9 and 4.4 µm, The percentages of BDP internalized and metabolized by Calu-3 cell monolayers from the two suspension formulations with different PSDs show no significant difference (Fig. 3).

Nose model to demonstrate *in vitro* nasal deposition

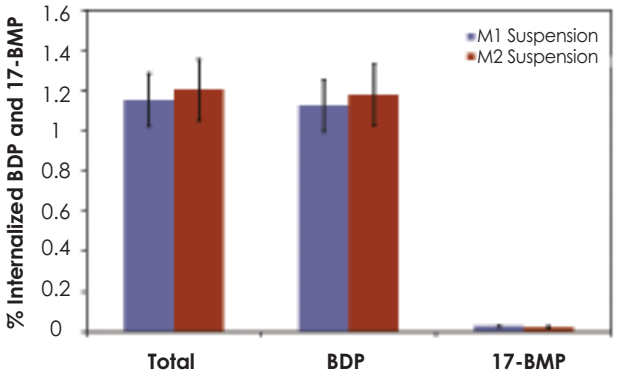
Evaluation of differences in nasal deposition location produced by a delivery device have typically used radioisotope volunteer studies, which provide useful information but require adulteration of the formulation with a radiolabel. Development of an *in vitro* model could provide faster analysis without any alteration to the formulation.

An anatomically correct, transparent, silicone human nose model in conjunction with a commercially available water level-indicating paste that changes color on contact with water provides a novel method of visualizing deposition of aqueous droplets within the nasal cavity. Using a digital camera to capture images and Adobe Photoshop to quantify the region of color change, analysts have documented the expected differences in deposition from Afrin nasal spray and the Sinustar nasal nebulizer after spraying each into a silicone nose model uniformly coated with Sar-Gel indicating paste (Fig. 4).

The images captured demonstrate a significant and quantifiable change in the color of the paste from white to purple as droplets or liquid streams from each nasal product come into contact with the Sar-Gel. In addition, the model proved consistent with previous scintigraphic studies in human volunteers

Figure 3

Percentages of BDP internalized and metabolized by Calu-3 cells incubated with BDP suspensions with different primary drug particle size distributions



that showed that the nasal nebulizer significantly increased deposition beyond the anterior nasal cavity [8]. The study indicated that the spray pump deposited droplets primarily in the anterior portion of the nasal cavity as expected due to the larger droplet size.

References

1. Chow, S.-C.; Shao, J.; and Wang, H. *In vitro* bioequivalence testing. Statist Med. 22: 55-68 (2003).

2. Cheng, B. and Shao, J. Profile analysis for assessing i*n vitro* bioequivalence. J Biopharm Stat. 12(3): 323-332 (2002).

3. FDA. US FDA Draft Guidance for Industry. Bioavailability and bioequivalence studies for nasal aerosols and nasal sprays for local action. Center for Drug Evaluation and Research, US Food and Drug Administration (2003).

Figure 4

Left: Silicone nose model with Sar-Gel after droplet deposition with nasal spray. Right: Silicone nose model with Sar-Gel after droplet deposition with nasal nebulizer

4. Dayal, P.; Shaik, M. S. and Singh, M. Evaluation of different parameters that affect droplet-size distribution from nasal sprays using the Malvern Spraytec. J Pharm Sci. 93(7): 1725-1742 (2004).

5. Pennington, J.; Pandey, P.; Tat, H.; Willson, J.; and Donovan, B. Spray pattern and droplet size analyses for high-shear viscosity determination of aqueous suspension corticosteroid nasal sprays. Drug Dev Ind Pharm. 34: 923-929 (2008).

6. Suman, J. D.; Laube, B. L.; and Dalby, R. Validity of *in vitro* tests on aqueous spray pumps as surrogates for nasal deposition, absorption, and biologic response. J Aerosol Med. 19(4): 510-521 (2006).

7. Guo, C. and Doub, W. H. The influence of actuation parameters on *in vitro* testing of nasal spray products. J Pharm Sci. 95(9): 2029-2040 (2006).

8. Suman, J. D.; Laube, B. L.; and Dalby, R. Comparison of nasal deposition and clearance of aerosol generated by nasal nebulizer and an aqueous spray pump. Pharm Research. 10: 1648-1652 (1999).

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