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IMPROVING *IN VITRO* TEST METHODS FOR NASAL DRUG PRODUCTS

In this article, Mark Copley, Chief Executive Officer, and Anna Sipitanou, Business Development Manager, both of Copley Scientific, look at how *in vitro* testing is being refined for greater repeatability and to maximise its value as a low-cost tool for product development, including an assessment of the role of automation in improving the sensitivity of *in vitro* testing.

There are sound reasons why *in vitro* test methods for nasal drug products are currently subject to scrutiny with a view towards their improvement, such as by shaping them to provide more precise information for targeted delivery. The nasal route is of growing interest for systemic drug delivery, in no small part due to the access it provides to the central nervous system and the associated potential to bypass the blood-brain barrier.¹ Nasal drug products for migraine already demonstrate this potential, which holds promise for the treatment of neurological conditions such as Alzheimer's disease, depression and schizophrenia. For therapies such as these, drug delivery to specific areas of the nasal cavity can be important. Nasal vaccine delivery is also now increasingly well-established, offering recognisable advantages for the delivery of vaccines for influenza, HIV and hepatitis.² Furthermore, trials for covid-19 nasal vaccines are already underway.³

On the other hand, nasal sprays for local action, such as those for the treatment of allergic rhinitis and hay fever, still dominate the market, making them an important target for generic manufacturers.

Pharmacokinetic studies have limited relevance when it comes to demonstrating bioequivalence for locally acting products,^{4,5} intensifying the need to optimise the application of *in vitro* techniques. Sensitive techniques with proven clinical relevance have a vital role to play in supporting abbreviated new drug applications.

NASAL DRUG DELIVERY – FROM GENERATION TO DEPOSITION

Many commercial nasal drug products are mechanical metered dose sprays, packaged either as a multi- or unit-dose format. Nasal aerosols that employ a propellant for drug delivery are an alternative for liquid formulations, along with nasal drops, which are a popular choice for locally acting therapeutics. Nasal powders have also been commercialised, as exemplified by Baqsimi (glucagon) (Eli Lilly), for the treatment of severe hypoglycemia.⁶ Closer examination of the mechanisms of drug delivery via nasal spray can help to elucidate the challenges associated with product development, many of which are common to all nasal drug products.

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Drug delivery via a nasal spray is driven by a manually actuated metering pump, the performance of which is manipulated via design parameters such as precompression ratio and the length, geometry and orifice size of the actuator. Nasal spray formulations are solutions or suspensions of one or more APIs, often in an aqueous-based system, with precisely controlled physical properties, notably their rheology. The properties of such formulations are optimised for stability and for dispersion by the shear force applied via actuation of the chosen device.

The patient is the final variable in the drug delivery process. Factors such as insertion depth into the nostril, orientation angle and the force/time profile applied during actuation all influence the characteristics of the delivered dose. Furthermore, there is the unique physiology of the patient's nasal cavity to contend with. The vasculature-rich turbinates are the typical target for drug delivery, however, there is the potential for very fine (<10 µm) droplets to penetrate through the nasal cavity and deposit in the lung, compromising drug safety, while larger droplets may deposit at the front of the nose, undermining efficacy. Droplet collection in the anterior cavity and subsequent dripping from the nostril or, conversely, an unpleasant taste after product use due to droplets reaching the nasopharynx, are both unwanted side effects of suboptimal drug deposition.

This brief examination of the factors influencing nasal drug delivery highlights the multifactorial space that must be robustly explored to develop a nasal drug product with consistent, reproducible and well-defined deposition characteristics. *In vitro* test methods generate vital information and are lower cost, less variable, easier to control and more reliably differentiating than clinical trials. However, they may lack clinical relevance. Scrutiny as to whether the precision,

relevance and application of current techniques is optimal is therefore a natural consequence of the desire for faster, more cost-efficient development.

ASSESSING THE PERFORMANCE OF NASAL DRUG PRODUCTS

In vitro tests recommended in US FDA guidance for nasal drug products include:⁵

- Single actuation content (alternatively referred to as dose uniformity) through the life of the container
- Droplet size distribution by laser diffraction
- Drug in small particles/droplets (for nasal sprays) or particle/droplet size distribution by cascade impaction (for nasal aerosols)
- Drug particle size distribution by microscopy (for suspension formulations)
- Spray pattern
- Plume geometry
- Priming and repriming.

These well-established tests provide a wealth of information to guide product development and/or demonstrate bioequivalence. Dose uniformity testing, for example, confirms the consistency with which the label dose is delivered across the lifetime of the product. Droplet size distribution has a defining influence on *in vivo* behaviour; droplet sizes in the range of 20–30 µm, in combination with low injection velocities, are associated with high levels of drug deposition in the turbinates,⁷ and the amount of drug in small particles is relevant to the potential hazard of pulmonary deposition. Priming and repriming tests assess how reliably and consistently the product works if used according to the instructions for use with respect to, for example, storage and shaking.

However, the standard methods and equipment have their limitations, especially when set against efforts to extend the application of nasal drug delivery, in particular with respect to the study of regional deposition. Supplementary techniques deployed for the study of regional deposition behaviour include testing with nasal casts or replicas and gamma scintigraphy, though neither is readily deployed in routine workflows. Important issues relating to the use of realistic nasal replicas include the manufacture of such replicas and the time-consuming, manually intensive nature of drug recovery, due to the complex geometry involved.

Gamma scintigraphy is a non-invasive imaging technique that involves incorporating radioactive tracers into a formulation and subsequently detecting their location, *in vivo*, following product use. With this technique, it is possible to generate confirmatory data that test and reference product deposits analogously, to support bioequivalence claims and to compare formulations and/or devices with respect to drug delivery to a specific target. However, again, gamma scintigraphy is far from a routine benchtop tool.

When it comes to shaping analytical tools to better support nasal drug product development, it is important to consider both the information being sought and the practicalities of routine measurement.

AUTOMATION AND ITS ROLE IN REDUCING CONFOUNDING VARIABILITY

Automation is a well-recognised route to easing the manual burden associated with routine analysis while simultaneously improving data quality. Just as patient technique is a source of variability in nasal drug delivery, manual spray actuation can erode the sensitivity of *in vitro* testing. Automating device actuation can therefore play a useful role in reducing variability and improving data integrity, as evidenced by the US Pharmacopeia (USP) specification that “a mechanical means of actuating the pump assembly be employed to deliver doses for collection” when testing nasal sprays.⁸

Reducing method variability enables better investigation of the variability that is actually of interest to drug developers – the variability that arises from differences relating to the device, formulation or patient. In the same way, reducing method variability improves the sensitivity of comparative studies in generic product development, making it easier to robustly detect differences between a test and reference product, or conversely to confirm equivalence more reliably.

Table 1 shows data from a single-actuation content study carried out using Vertus II, Copley Scientific's automated system for nasal spray actuation, in combination with Copley's Nasal Spray Dose Collector (NSDC), an innovative accessory for dose sampling. Crucial to any testing method is the complete and demonstrable capture of the delivered dose. However, sample loss due to dripping and leakage is commonplace, due to the need to

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fire nasal sprays upwards to simulate actual product use coupled with the widespread use of glass flasks or vials for dose collection.

Copley's NSDC has a curved impaction surface that minimises dripping onto the spray nozzle tip and an internal geometry that guides the collected sample to a small collection sump. The Vertus II interfaces directly with the NSDC, fully automating actuation and test flow control. The speed, angle and duration of shaking ahead of actuation; firing force, including the speed of application and release of that force; and time delay between shaking and firing can all be defined and closely controlled, in accordance with test requirements.

In the single actuation content study, testing was carried out using a commercial beclomethasone dipropionate formulation filled into a standard commercial nasal spray pump/bottle (Aptar Pharma). The delivered dose was determined by opening the NSDC and recovering the drug via a process of rinsing and sonication. Drug was also recovered from the nasal spray nozzle via rinsing and dissolution. The total metered dose was determined by summing the drug recovered via both routes and then compared with the label claim. The results showed extremely high repeatability across a series of ten tests, far higher than the 75–125% acceptability limits specified in the European Pharmacopoeia.⁹ As discussed, such precision translates into high sensitivity, enabling studies to differentiate between products more accurately.

Figure 1 shows an alternative test set-up for automated dose delivery measurement using a standard metered dose inhaler (MDI) dose uniformity sampling apparatus (DUSA). Recent revisions to the European Pharmacopoeia and USP <601> updated guidance on delivered dose uniformity for nasal sprays, aligning it with guidance for pressurised MDIs.^{8,9} Direct interfacing of the DUSA with the Vertus II provides a simple, easy-to-use and high integrity set-up for automated testing.

A BENCHTOP TOOL FOR ASSESSING REGIONAL DEPOSITION

Recognition of the potential benefit of a better benchtop tool for investigating regional deposition for nasal drug products has led to the development of the Alberta Idealised Nasal Inlet (AINI),¹⁰ using analogous methodologies to those deployed in the development of the Alberta Idealised Throats. Both products

NSDC ID	DD NSDC (µg)	Nozzle (µg)	MD (µg)	Recovery (MD % of label claim)
NSDC 1	49.909	0.224	50.13	100
NSDC 2	48.567	0.494	49.06	98
NSDC 3	49.944	0.330	50.27	101
NSDC 4	49.125	0.431	49.56	99
NSDC 5	47.781	0.453	48.23	96
NSDC 6	49.679	0.458	50.14	100
NSDC 7	49.726	1.168	50.89	102
NSDC 8	43.359	0.312	43.67	87
NSDC 9	49.320	2.597	51.92	104
NSDC 10	49.044	0.535	49.58	99
Mean (±SD)	48.65 (± 2)	0.7 (± 0.7)	49.35 (± 2.2)	99 (± 4)

Table 1: Dose uniformity testing for a beclomethasone dipropionate nasal spray with automated actuation (Vertus II) and NSDC for dose collection.

were originally developed by researchers working with Professor Warren Finlay at the University of Alberta (Edmonton, Canada) and subsequently commercialised by Copley Scientific. Rather than realistic replicas of the nasal cavity or throat, these “idealised” accessories are easy-to-use tools that accurately mimic deposition behaviour for a wide range of patient physiologies.

Development work for the AINI began with computational fluid dynamic simulations of drug deposition behaviour in realistic nasal airway geometries

derived from computer tomography (CT) scans of seven adult subjects.⁷ Close to 250,000 data sets were produced to rigorously assess the impact of various parameters, including droplet size and injection velocity, for sprays injected separately in both left and right nostrils. An idealised geometry was developed from these data,^{6,9} striking a balance between an accurate representation of the deposition behaviour observed in the replicas and a simple, easy-to-use solution (Figure 2).



Figure 1: A test set-up for dose uniformity testing with automated actuation (Vertus II) and MDI DUSA.

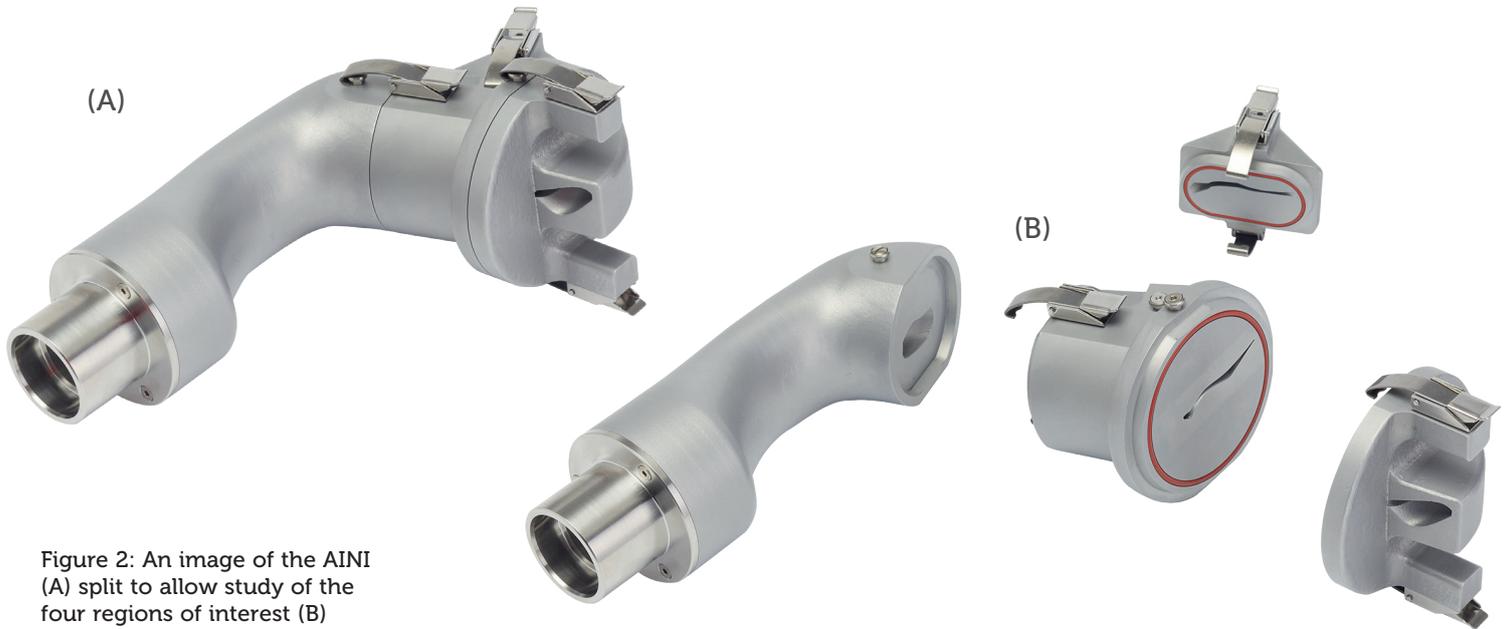


Figure 2: An image of the AINI (A) split to allow study of the four regions of interest (B)

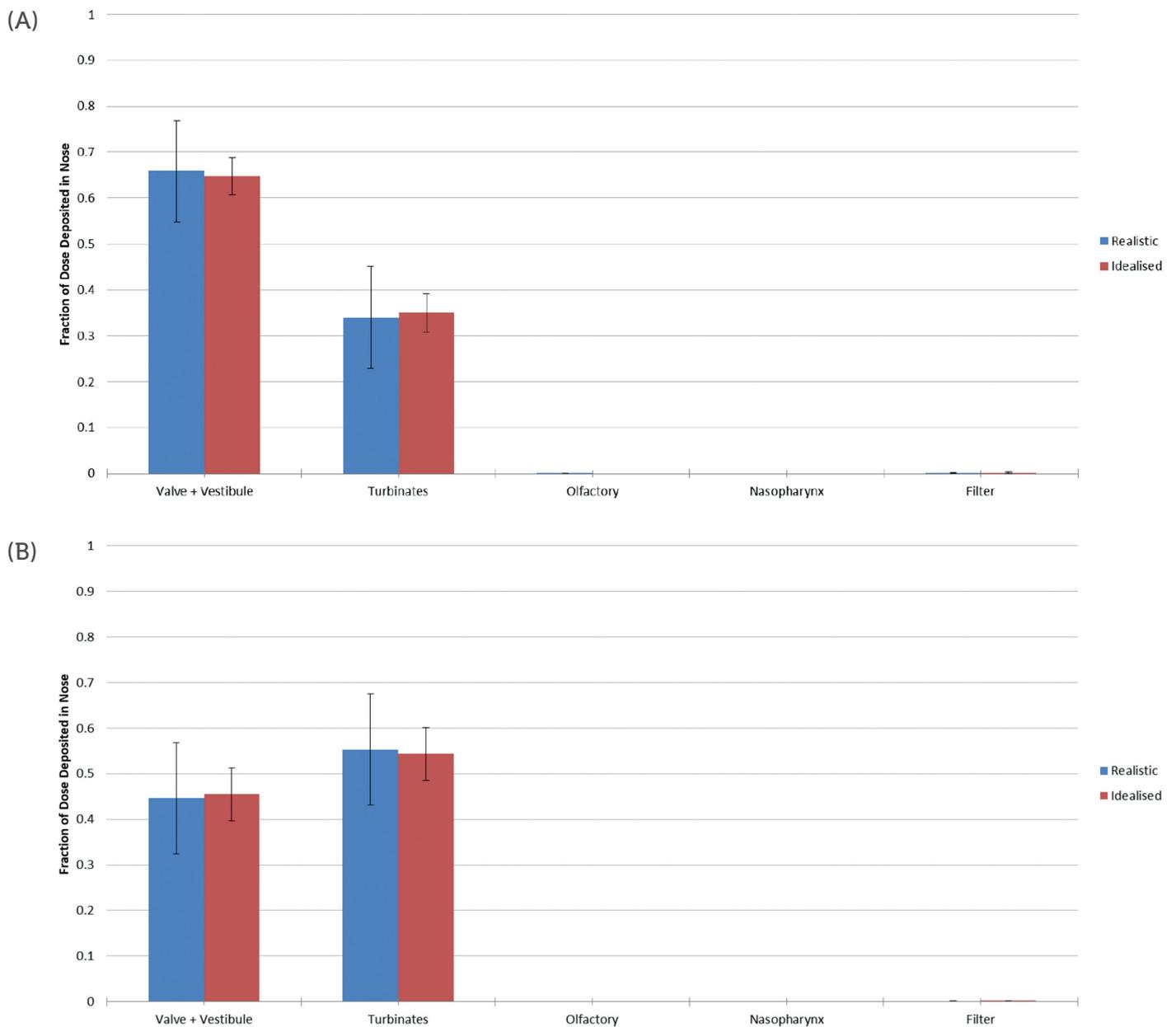


Figure 3: Deposition data for a commercial nasal spray, at an orientation angle of 60° (A) and 45° (B), highlight the ability of the idealised geometry to accurately reflect behaviour in realistic nasal geometries and reduce variability.

Figure 3 shows data from an experimental study of the performance of the idealised geometry relative to realistic nasal replicas.^{11,12} In this work, five polymeric nasal replicas were manufactured from CT data gathered from adult subjects, using a rapid prototyping technique. These were then used to assess drug deposition from a commercial nasal spray pump (specifically a 5.2 mg cromolyn sodium spray). Testing was carried out at an inspiratory flow rate of 7.5 L/min at two different orientations: 60° and 45° from horizontal. Comparative testing was also carried out with a rendering of the idealised geometry produced using the same prototyping technique. Note that, in this study, the element of the idealised geometry referred to as “vestibule” in the AINI is referred to as “vestibule + valve” for comparison with the nasal replicas.

The results showed close agreement between the data generated using the idealised geometry and the realistic nasal replicas. However, the results generated with the idealised geometry exhibited far less variability, enhancing their value for scientific studies. Interestingly, the data gathered at different orientations showed a significant difference in deposition behaviour, underlining the importance of this patient technique-related variable in the drug delivery process.

For this nasal spray there are also published gamma scintigraphy data quantifying *in vivo* deposition in five male subjects.⁴ In this work, average deposition in the anterior region, which translates approximately to the valve + vestibule, was found to be $0.56 \pm 0.3 \mu\text{g}$, while posterior deposition, which can be equated with deposition in the turbinates, was $0.44 \pm 0.1 \mu\text{g}$. The administration angle was not reported in this work, but this compares with 0.45–0.65 μg and 0.35–0.55 μg respectively, depending on orientation (Figure 4). In both studies deposition in the nasopharynx was found to be negligible.

These results confirm the relevance and utility of the AINI which, in practice, has two key applications. Firstly, it can be attached directly to an external filter to directly investigate regional deposition, as exemplified by this study, as well as to representatively measure respirable mass – the mass of dose that may reach the lung. Such studies are valuable for targeting drug delivery in new drug development and for the demonstration of bioequivalence in generic product development. The second application is to interface the AINI with

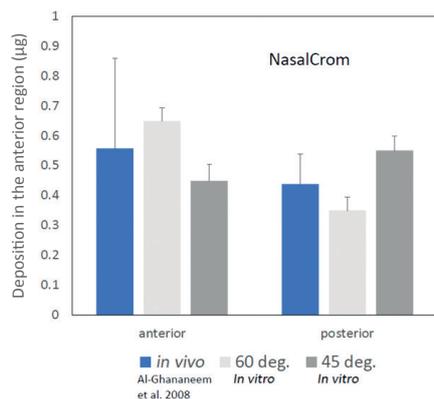


Figure 4: Deposition data for a commercial nasal spray measured with the idealised geometry show good agreement with *in vivo* gamma scintigraphy data.

a cascade impactor to representatively determine the amount of drug in small particles/droplets with a higher degree of clinical realism. An inlet that fails to simulate drug capture in the nasal cavity prevents representative assessment of the respirable dose.

GOING FORWARD

Better solutions for *in vitro* testing have an important role to play in accelerating the development of nasal drug products, whether the goal is new vaccines for covid-19, more clinically efficacious therapies for neurological diseases or cost-effective products for everyday ailments that negatively impact quality of life. Automation can reduce the variability associated with testing, sharpening sensitivity while also boosting analytical productivity. On the other hand, benchtop tools for the study of regional deposition are a cost-effective option for researchers investigating how to target specific areas of the nasal cavity to enhance drug delivery, as well as for those looking to support claims of bioequivalence. For new and generic nasal product developers alike, these tools are an important step forwards in efforts to exploit the full potential of the nasal route for drug delivery, and realise the exciting possibilities it promises.

ABOUT THE COMPANY

Copley Scientific is recognised as a leading manufacturer of inhaled drug test equipment. Products include delivered dose-sampling apparatus, Andersen and Next Generation impactors, critical flow controllers, pumps, flow meters and inhaler-

testing data analysis software. Copley Scientific also supplies novel systems for improving productivity and *in vitro/in vivo* correlations, including semi-automation ancillaries, abbreviated impactors, breath simulators and the Alberta Idealised Throats. Training, calibration, maintenance and impactor stage mensuration services are also available. Founded in 1946 in Nottingham, UK, Copley Scientific remains family owned and managed. The company continues to work closely with industry groups and leading experts to bring relevant new products to market, with all equipment backed by expert training and lifetime support.

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ABOUT THE AUTHORS

Mark Copley graduated from the University of Bath, UK, in 2000 with a masters degree in Aerospace Engineering. For eight years he was Technical Sales Manager and Product Specialist for Copley Scientific's range of inhaler testing equipment, before becoming the Sales Director in 2009. Mr Copley is now Chief Executive Officer for the company. Mr Copley is considered a leading authority in testing methods and systems for MDIs, DPIs, nebulisers and nasal sprays; authoring and contributing to more than 50 published articles. He also provides application support and consultancy, runs focused training workshops for the inhaled drug testing sector of the pharmaceutical industry and sits on the editorial advisory panel of Inhalation magazine. An invited member of the European Pharmaceutical Aerosol Group impactor sub-team, Mr Copley has also made recommendations to the Inhalanda working group, leading to subsequent revisions to Ph Eur and USP monographs. As part of Copley Scientific's associate membership of the International Pharmaceutical Aerosol Consortium on Regulation & Science, Mr Copley participates in a number of working groups with a view to enhancing the regulatory science of orally inhaled and nasal drug products (OINDP).

Anna Sipitanou holds a BSc in Chemistry and an MSc in Drug Discovery & Pharmaceutical Sciences. Having joined Copley Scientific in 2017, Ms Sipitanou plays a key role in the company's technical and sales support services, including the training of customers on a wide range of pharmaceutical testing equipment, with a particular focus on OINDP testing. Having worked closely with pharmaceutical companies on a wide range of OINDP projects, Ms Sipitanou has gained specialist knowledge of the regulatory requirements for both delivered dose uniformity and aerodynamic particle size distribution testing, as well as extensive experience in methods to improve *in vitro/in vivo* correlations and other specialist testing applications, including generic drug development, inhaled dissolution and facemask testing.

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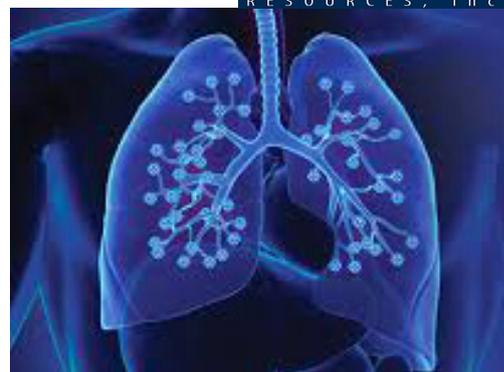
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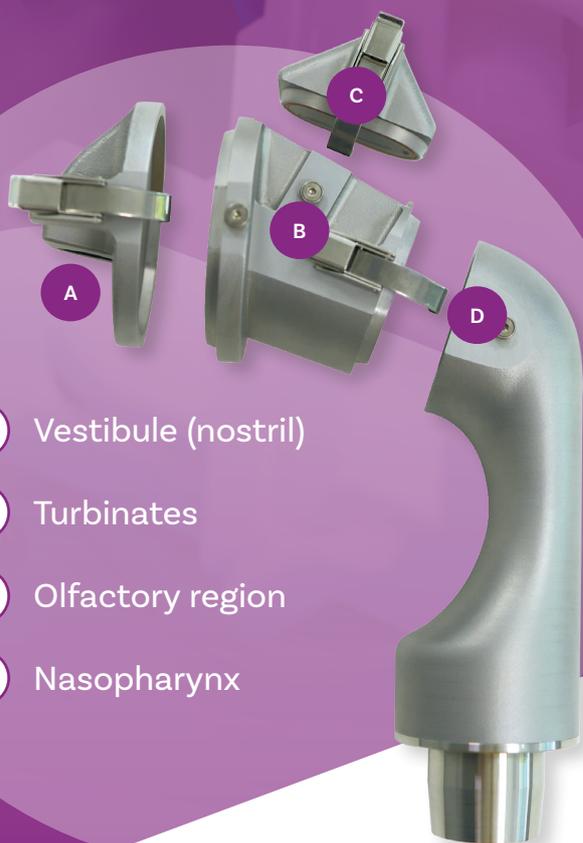
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