SEMI-AUTOMATION IN INHALER TESTING – EXPLORING THE POTENTIAL AND PRACTICALITIES

In this article, João Pereira, Team Leader R&D Analytical Development, and Raquel Borda D'Água, Associated Analytical Chemist, both at Hovione, and Mark Copley, Chief Executive Officer, and Anna Sipitanou, Business Development Manager, both at Copley Scientific, discuss the semi-automation of cascade impactor testing and the benefits it can bring in terms of quality of orally inhaled product test data, and reductions in cost and effort associated with routine, critical measurements.

INTRODUCTION

The automation of discrete steps of cascade impactor analysis offers opportunities to address variability in inhaler testing, while simultaneously reducing health and safety concerns and improving analyst productivity. The ability of cascade impaction to generate drug-specific aerodynamic particle size distribution data (APSD) for orally inhaled products (OIPs) is central to its utility, but necessitates systematic drug recovery from each stage of the impactor, and from the surfaces of other accessory components that complete the test set-up. This laborious task accounts for much of the manual effort associated with cascade impaction measurements and is a primary focus for automation. The rewards can be significant; however, such changes raise questions of equivalence to manual methods, which must be robustly answered prior to the adoption of automated methodologies.

In this article, we consider the semi-automation of cascade impactor testing focusing on those tasks, notably aspects of drug recovery that are easily tackled using off-the-shelf solutions. A back-to-back comparative study of manual and automated drug recovery carried out by Hovione, a leading contract development and manufacturing organisation, demonstrates statistical equivalence between the methods and highlights a reduction in analyst bench time of about 40%.

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THE CASCADE IMPACTION WORKFLOW

A cascade impactor is a precision instrument that fractionates a sample on the basis of particle inertia, which is a function of particle size and velocity. The workflow associated with producing drug-specific aerodynamic particle size distribution (APSD) data for an OIP can therefore be split into two discrete elements: size fractionation of the dose (by the impactor) followed by drug recovery and quantitation, to determine the drug deposition profile.

The cascade impactor test set-up for any specific application is defined with reference to the device under test and the purpose of analysis, for example, whether the aim is to generate more clinically realistic data for research and product development, or

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to confirm batch-to-batch consistency for product release. A detailed discussion of cascade impactor test set-up and the issues associated with method development lies beyond the scope of this article, but is well covered by Bonam et al (2008).[1]

Once a test set-up has been established, routine analysis is initiated by actuating the device to release a dose into the impactor. A vacuum pump draws the sample-laden air through the stages of the impactor at a constant, defined volumetric flow rate, causing the deposition of particles above a certain cut-off diameter on the collection surface of each stage; each subsequent stage captures progressively smaller particles. Multiple actuations are frequently required to ensure a quantifiable amount of drug on each collection surface and to guarantee method repeatability.

At the end of this first part of the analysis, multiple doses of the drug product are distributed, depending on the exact test set-up, across: the mouthpiece adaptor (MA), the induction port (IP) – the interface between the device and the impactor – the pre-separator (PS) when used, each stage of the impactor, and the micro-orifice collector (MOC) or final filter.

Completion of the analysis involves the rigorous recovery of samples from each of these surfaces. This involves wetting and rinsing each surface to dissolve the deposited sample with a suitable solvent and produce solutions of an adequate concentration for assay, typically via liquid chromatography (LC). The resulting data are converted into APSD metrics specifically for the API, typically using dedicated software.

The product-specific nature of cascade impactor test set-ups and the complexity of the measurement process directly influence the feasibility of end-to-end automation, which is rarely, if ever, cost effective. Conversely, automating specific steps with off-the-shelf solutions can be highly beneficial. The cost of such solutions is far more accessible than a bespoke automation project and they can deliver significant improvements in day-to-day practice, reducing analyst fatigue and stress, and the risk of repetitive strain injury (RSI) by eliminating time-consuming repetitive tasks. Critically, automation can improve data quality, accuracy and integrity by eliminating the effect of operator-to-operator variability and handling errors.

For many organisations, the number of samples lost due to simple but impactful handling errors is significant and results in, at best, repeat analyses and, at worst, a costly, time-consuming investigation. For example, automated shake-and-fire systems ensure highly repeatable device actuation in metered dose inhaler (MDI) testing by applying a consistent, well-defined device use regime (between actuations), shaking protocol and actuation force profile. This can help to significantly reduce variability in the delivered dose and, by extension, the whole measurement.[2] More generally, for all OIPs it is the process of drug recovery that is most amenable to automation, with off-the-shelf solutions ranging from simple rinsing devices through to sophisticated systems for complete automation.

FOCUSING ON DRUG RECOVERY

Developing a robust, optimised method for drug recovery involves the careful consideration of issues such as:

- Which solvent is most appropriate – while highly volatile solvents may be essential to achieve complete dissolution, solvent evaporation can compromise pipetting and the delivery of accurate solvent volumes. Furthermore, volatility enhances the risk of sample concentration due to solvent loss during storage or the drug recovery process.
- How much solvent should be used – high solvent volumes ease complete drug dissolution by improving sink conditions, but simultaneously reduce drug concentration, potentially compromising the accuracy of the assay. Wide variation in the amount of drug that deposits on any given stage of the impactor can make it difficult to ensure complete dissolution of the drug at high loadings while simultaneously ensuring that the sample has a concentration above the limit of detection (LOD)/ limit of quantification (LOQ) for stages on which drug deposition is minimal. This issue can be especially challenging for products with more than one active ingredient. There is also a positive environmental impact in lowering solvent content for extraction purposes.
- The best method to promote rapid and effective drug dissolution – to ensure complete dissolution, the drug and solvent must be in contact for an adequate length of time. Agitation accelerates dissolution and helps to ensure complete surface wetting; the application of ultrasonics is an option for less easily dissolved actives.
- What equipment to use to minimise sample degradation – any container in which recovered drug solutions are going to be held, including vials used for analysis, requires careful consideration to avoid, for example, sample loss to vial walls, absorption of the active from the solution and/or solvent evaporation.

A validated drug recovery method may be entirely manual but, where this is the case, analysis will necessarily involve a number of repetitive activities that are either significantly prone to error or physically arduous, or indeed both. Prime examples include pipetting and agitation of a specific test component with a defined aliquot of solvent. With these tasks, even simple devices, such as automated pipettes or rocking/rinsing devices, can make a major difference. For example, the Sample Preparation Unit Model SPU 2000 automates internal rinsing of the USP/PhEur induction port and the Next Generation Impactor (NGI) pre-separator, delivering consistent wetting of the internal surfaces and reproducible dissolution via the application of a defined agitation pattern for a set period of time.

Semi-automation with simple devices of this type is typically low cost and low risk, and the economic payback can be attractive, with analysts freed for higher value activities. On the other hand, more sophisticated off-the-shelf solutions, such as the NGI Assistant, can prove an even more beneficial investment over the long

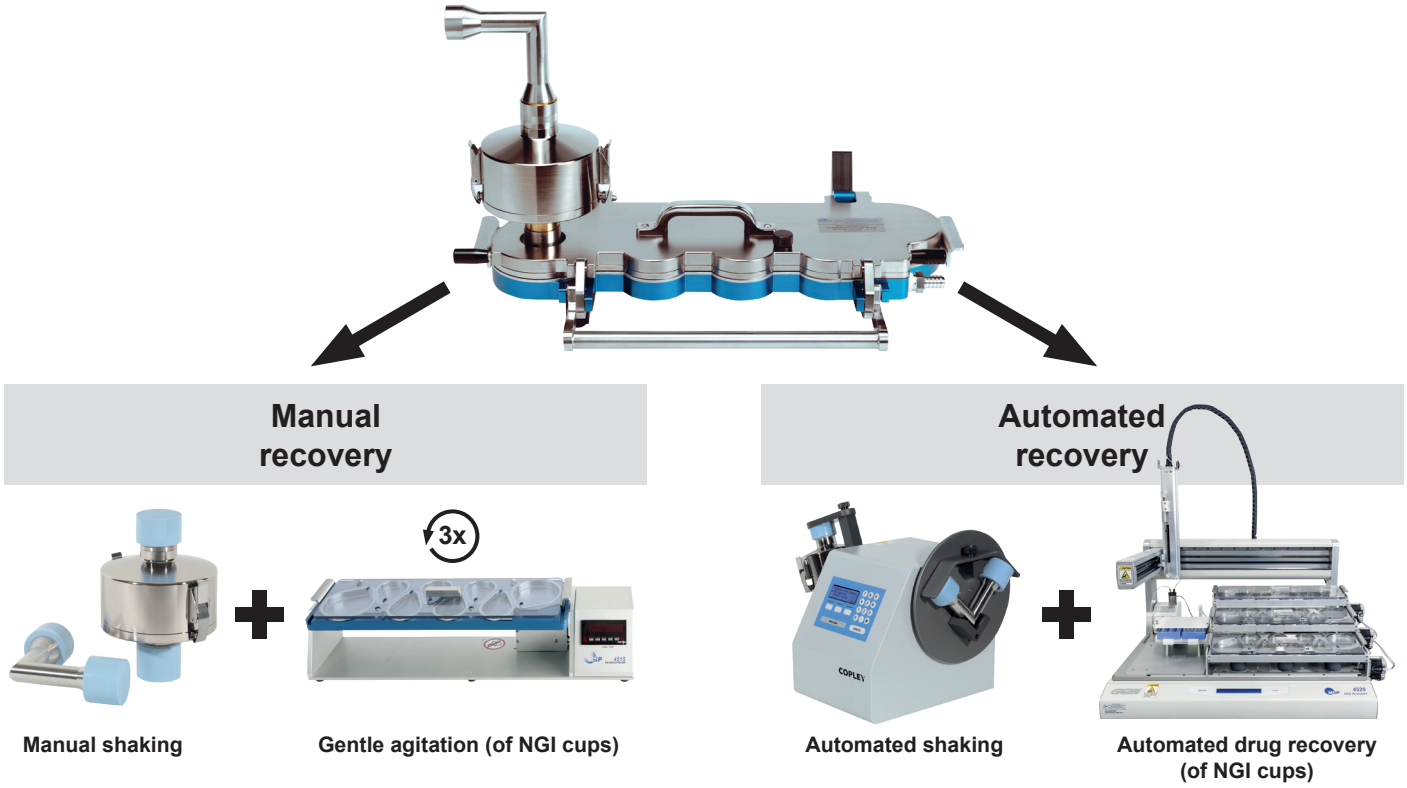

Figure 1: Copley Scientific equipment was used in many of the manual and automated drug recovery workflows in the study.

term. Systems which automate multiple steps of the drug recovery process may be associated with higher capital expenditure but can deliver more substantial gains by simultaneously addressing multiple sources of measurement variability. The NGI Assistant automates drug recovery from the point of solvent dispensation and drug dissolution through to the presentation of sample solutions in industry-standard vials, ready for liquid chromatography (LC) analysis, thereby eliminating any requirement for manual pipetting, agitation or LC sample preparation.

In the following study, predominantly manual analysis was compared with more fully automated analysis using this system to demonstrate a) the time savings are accessible and b) whether the data generated are strictly equivalent.

CASE STUDY: COMPARING MANUAL AND SEMI-AUTOMATED DRUG RECOVERY FOR CASCADE IMPACTOR TESTING OF A DPI

APSD data for a TwinCaps® single-use dry powder inhaler (DPI) were generated using two different methods for drug recovery (Figure 1): an essentially manual recovery method aided by an automated solution for agitation of the solvent in the NGI collection cup tray (NGI Gentle Rocker) and a fully automated recovery with an NGI Assistant. Testing was carried out using an in-house method developed in accordance with the relevant general chapter of the PhEur.[3] An NGI with USP/PhEur induction port and pre-separator was used with a test flow rate of 38 L/min, determined on the basis of a 4 kPa pressure drop across the device. A mixed solvent was used for drug recovery (details not specified) and the resulting solutions were quantified using an HPLC system (MA, US). HPLC was carried out using a silica-based column with a mixed aqueous and organic mobile phase (flow rate 0.8 mL/min) and an injection volume of 100 µL.

HPLC data were analysed using Empower 3 software (MA, US). CITDAS software (Version 3.10) was then used to generate APSD metrics for the inhaler including fine particle dose (FPD), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). A total of 23 replicate tests were carried out in total, 10 by Analyst One using the manual drug recovery method, three by Analyst Two using the same method, and 10 using the more automated method. Mass balances checking (referencing a label claim of 135 µg) confirmed that all runs fulfilled the relevant mass balance criteria: emitted dose (ED) lying between 75% and 125% of label claim.[3] Equivalency between the datasets was assessed via t-testing, a statistical method for determining the extent to which two datasets are identical.

Table 1 shows the percentage variance in the amount of drug recovered from each stage of the impactor for the runs carried out by Analyst One alone and for the two analysts combined. These data illustrate how, in general, variability increases when measurements are carried out by multiple analysts. This intuitive

	Analyst 1	Analyst 1 and 2
Stage 1	0%	10%
Stage 2	3%	14%
Stage 3	6%	7%
Stage 4	4%	3%
Stage 5	1%	2%
Stage 6	2%	2%
Stage 7	2%	2%
MOC	1%	2%

Table 1: Percentage variability in the mass (µg) recovered from each stage of the impactor by Analyst 1, and by Analyst 1 and 2 combined.

	p-value	t-Stat	t-Critical
Stage 1	0.28	-1.12	2.10
Stage 2	0.38	-0.89	2.08
Stage 3	0.61	-0.51	2.08
Stage 4	0.36	0.94	2.11
Stage 5	0.33	1.00	2.12
Stage 6	0.28	1.12	2.14
Stage 7	0.73	-0.35	2.10
MOC	0.19	-1.36	2.12

Table 2: Comparing the equivalence of manual and automated drug recovery.

	p-value	t-Stat	t-Critical
FPD	0.46	0.76	2.10
MMAD	0.39	-0.88	2.09
GSD	0.21	-1.31	2.09

Table 3: Critical metrics generated using manual and automated drug recovery methods were shown to be statistically equivalent.

finding stems from the fact that manual analyses are inherently subject to both intra- and inter-operator variability; where more than two operators are responsible for analysis, variance might reasonably be expected to be even higher. Furthermore, the impact of operator variability is likely to be higher with a completely manual method, in the absence of the automated solution for solvent agitation. Since it would be rare for analysis for a given product always to be carried out by a single analyst, the combined Analyst One and Two dataset was selected as the more realistic basis for comparison of the impact of switching to automated drug recovery.

Table 2 shows t-test data (two-sample, unequal variances) for a comparison of the results produced by the established, more manual method (Analyst One and Two) and via automated drug recovery. When automating a manual method, it is crucial to confirm that the results are equivalent using statistical methods such as these. In the absence of such cross-validation, systematic differences can be introduced that ultimately result in the product failing to meet specification. Here, analysis shows that, for every stage, the absolute value of the t-statistic lies below t-critical and the associated p-value is well above the threshold value of 0.05, confirming statistical equivalence. Robust equivalence is also observed in the APSD parameters generated by each method (Table 3).

Monitoring analyst bench time during the study enabled calculation of the productivity gains accessible by switching to the more automated method. The results indicate that analyst bench time is reduced by around 40%, a substantial increase in productivity. The study also provides a good illustration of the potential to progressively adopt automation solutions, with cross-validation at each stage reducing the risk of introducing systematic differences.

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CONCLUSION

The semi-automation of cascade impaction has an important role to play in improving the quality of OIP test data, while at the same time reducing the cost and effort associated with routine, critical measurements. This study illustrates how sophisticated, easy-to-use, off-the-shelf solutions can be used to generate statistically equivalent data and deliver major productivity gains, reducing analyst bench time by around 40%. By freeing analyst time for more valuable, less repetitive tasks, such solutions can help to minimise the risk of out-of-specification results, address health and safety concerns, and, at the same time, deliver an attractive return on investment.

ABOUT THE COMPANIES

Hovione is a specialised, fully integrated CDMO able to support drug substance, drug product intermediate and drug product at the same production site. With an exceptional regulatory track record, including the FDA, and 60 years of experience, the company offers a broad range of process and drug product development services. From small molecule API manufacturing to formulated drug product delivery, Hovione seamlessly integrates all drug product development phases, from small-scale feasibility studies to commercial-stage production. Hovione has focused resources into continuously developing expertise in particle design and formulation development for highly sophisticated inhalation APIs. From API to crystal, particle to powder blend, capsule to inhaler – the company masters every development step. Hovione also offers a full range of simple, patented, cost-effective DPIs (disposable, capsule, blister and large dose DPIs).

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

IVIVCs, including semi-automation, 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

João Pereira joined Hovione in 2015 as part of the R&D Drug Product Development – Analytical Development group, where he worked on analytical method development for new drug products and drug product intermediates. In 2017, Mr Pereira became lead analytical chemist supporting several development programmes of drug product and intermediate drug product. In 2019, he became team leader in Analytical Development, where analytical method development in the fields of physical and performance characterisation are performed. Mr Pereira holds an MSc in Pharmaceutical Sciences from the Faculdade de Farmácia da Universidade de Lisboa (FFUL). He has previously worked in the pharmaceutical chemistry field in the Research Institute for Medicines (iMed.ULisboa) and in a quality control unit in Laboratórios Vitória.

Raque Borda D'Água joined Hovione in 2018 as part of the R&D Drug Product Development – Analytical Development group, where she worked on analytical method development for new drug products and drug product intermediates. In 2019, Ms Borda D'Água became an analytical chemist in Hovione R&D Analytical Development, focusing on performance and physical characterisation methodologies to support several development programmes. Prior to Hovione, Ms Borda D'Água worked as a Research fellow at Cenimat|i3N in Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa (FCT-UNL), integrated into several projects focused on the design and build of microfluidic systems and materials characterisation. Ms Borda D'Água holds a BSc and a MSc in Biochemistry from FCT-UNL. Her Master's thesis focused on impregnation development techniques of low-cost zinc oxide nanoparticles with antibacterial properties in fabrics performed in Cenimat|i3N in collaboration with the Department of Science and Technology of Biomass.

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 PhEur and USP monographs. As part of Copley Scientific's associate membership of the International Pharmaceutical Aerosol Consortium on Regulation & Science (IPAC-RS), 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 OINPD 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 (IVIVC) and other specialist testing applications, including generic drug development, inhaled dissolution and facemask testing.