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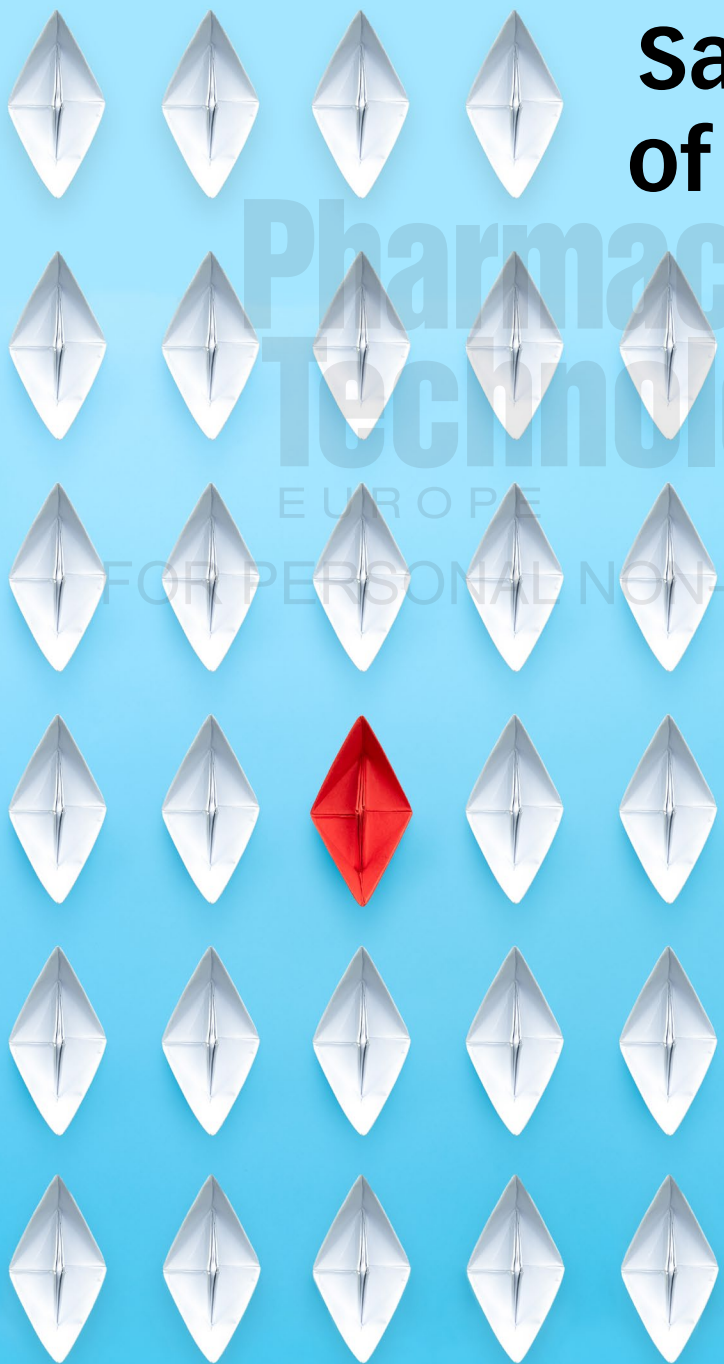

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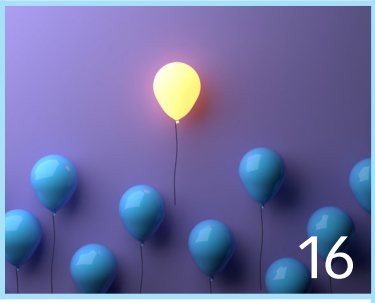

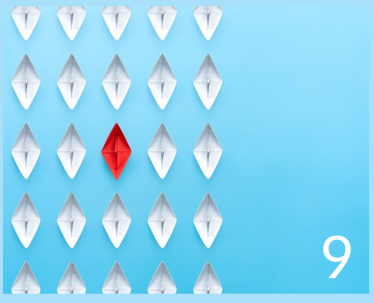

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Can We Teach an Old Drug New Tricks?

It has been widely reported, perhaps disproportionately due to the attentions of certain key political figures and the media, that there may be the potential for chloroquine and hydroxychloroquine to be used in the treatment of COVID-19 symptoms. However, there is little evidence to support efficacy of the drugs in the treatment of COVID-19, and, in fact, concerns are being raised about potential safety issues relating to the side effects of the therapies.

Serious risks and lacking evidence

Chloroquine and hydroxychloroquine were initially used as prophylaxis and treatment of malaria. More recently, these drugs have also been used in the management of other conditions, such as lupus and rheumatoid arthritis. However, these therapies are known to carry the risk of serious side effects, such as heart rhythm problems, liver and kidney problems, nerve cell damage, and low blood sugar.

In a press release, the European Medicines Agency (EMA) issued a reminder on the risks that are known to be associated with chloroquine and hydroxychloroquine (1). Additionally, the agency stressed that there is a chance the side effects can be exacerbated if these therapies are combined with other medicines, such as azithromycin—an antibiotic that may result in similar side effects on the heart (1).

Many of the studies that support the use of chloroquine and hydroxychloroquine to treat COVID-19 are preprints (not yet subjected to peer-review and/or are not necessarily suitably designed to determine effectiveness), have included only small patient populations, and are methodologically flawed. The French study that piqued interest in using hydroxychloroquine to treat COVID-19, by way of example, was not designed to the expected industry standard and, so despite being published (2), is now undergoing an additional independent peer review (3).

Furthermore, studies demonstrating a contrary opinion—that chloroquine and hydroxychloroquine do not have a suitable risk-to-benefit ratio in terms of COVID-19 treatment—also have limitations. For example, a Brazilian study, which has been posted online as a preprint, has indicated an elevated risk of mortality and arrhythmias when administering a high dose of hydroxychloroquine (4). However, this study did not include a control group, involves simultaneous use of other drugs, includes severely infected patients, and only included a small patient population.

Clear benefits of repurposing

There are clear benefits to following a reformulation/repurposing pathway, particularly when time is of the essence, as is the case for COVID-19. As more expertly discussed in one of this month's 'Development' feature articles (on pages 16–18), reformulation can give developers a significant 'head-start' by minimizing regulatory risk and accelerating speed-to-market. Yet, it is also imperative that there is a benefit, and certainly no compromise, to the patient with the reformulated or repurposed drug.

On a positive note, in the current global pandemic, there are studies underway, that are designed appropriately (randomized, placebo-controlled, double-blind, etc.) and are expected to give more definitive results in terms of safety and efficacy of chloroquine and hydroxychloroquine to treat COVID-19 (5–7). So, to answer the question of whether it is possible to teach old drugs new tricks, then yes, it is possible, but it certainly should not be rushed, and appropriate, robust data should be ascertained to ensure treatment efficacy and patient safety, irrespective of how urgent the situation may be.

Stay safe and healthy.

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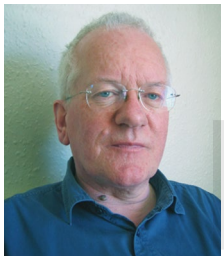
Can EMA's Regulatory Science Strategy Meet Medicine's Major Challenges?

EMA's strategy for regulatory science has divided opinion amongst various industry bodies.



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The European Medicines Agency (EMA) published, at the end of March 2020, the final version of a report on its strategy for regulatory science, which it sees as the basis for meeting the major challenges in medicine during the next five years (1). Three years of the report's preparation involved several rounds of consultation and two workshops with representatives of stakeholders.

But, unfortunately, the document was issued at a time when the coronavirus outbreak (COVID-19) had not yet shown itself to be causing the biggest public health crisis in Europe for 100 years. As a result, the report, which covers both human and veterinary medicines, devotes relatively limited space to ways of using regulatory science to deal with pandemics such as COVID-19, particularly the emergency development and mass-scale manufacture of vaccines.

Instead, to deal with the outbreak, EMA has implemented its 2018 Health Threat Plan by setting up a COVID-19 EMA Emergency Task Force (ETF), which will assist in the development, authorization, and safety monitoring of therapeutics and vaccines to deal with the pandemic (2). The agency has also been internationally active by co-chairing virtual meetings of the International Coalition of Medicines Regulatory Authorities (ICMRA) on collaboration between regulators on the development of vaccines and the authorization of repurposed drugs for COVID treatments (3).

Analysis of responses

EMA also published, with the regulatory science report, an analysis of stakeholder responses during the six-month public consultation to mid-2019 on the last draft of the document (4). The analysis confirmed that prior to COVID-19, pandemics and similar emergencies were not only being given relatively low importance by regulators but also by many stakeholder groups as well, particularly the pharma industry (4).

The study on responses also showed that among some stakeholders, even among national regulators, there is scepticism about EMA as the European Union's central agency for pan-European approval of drug taking on extended roles beyond issues of medicines authorization. This opposition may become significant if EMA's responsibilities are re-examined post-COVID-19.

EMA's consultation analysis, in which the 154 respondents, mostly in human medicines, were split into five clusters of patient/consumer organizations, healthcare professionals, research, public bodies, and the pharma industry, showed a relatively low recognition of the importance of issues linked to emergencies (4). These issues included health threat and preparedness planning, vaccines development and production, and repurposing of existing drugs. Of the regulatory science report's total of 30 core recommendations, these issues were all ranked among the lowest five in importance (4).

When respondents were asked to select one of their top three recommendations as delivering "the most significant change in the regulatory system over the next five years," one on developing a framework for repurposing drugs and another on health threat and preparedness plans received no support (4). A proposed initiative on "innovative approaches to the development and post-authorization monitoring of vaccines" was backed by only four respondents (4).

Emerging challenges

The regulatory science report highlighted the challenge of emerging technologies, such as new vaccine technologies and their manufacturing processes. But the main technologies pinpointed by the report were big data, precision medicines, clinical trial design, and even synthetic biology. For regulators, a big dilemma is the acquisition of knowledge about new sciences.

On plans for the emergency development of vaccines and their large-scale manufacture, EMA suggested working with the EU's regulatory partners outside Europe to harmonize the regulatory framework for vaccine clinical trials. Dedicated regulatory science

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advice and evaluation procedures have already been set up in the EU to support the fast development and oversight of new medicines, ranging from pandemic flu to any currently unidentified pathogen or 'disease X', the report said (1). The EU has research programmes to promote large-scale clinical research into infectious diseases and to design manufacturing processes to ensure rapid delivery of vaccines and antibodies.

According to the report, experiences with the Ebola and Zika viruses, as well as the COVID-19 coronavirus, have demonstrated the importance of communications within the EU regulatory network and among stakeholders. To help preparedness, scientific evidence should be defined prior to outbreaks so that resources needed to combat the disease can be identified and ring-fenced (1). Additionally, the report suggested the creation of a platform for the monitoring of vaccine safety and effectiveness in the post-approval phase. It also recommended fostering the development of improved delivery systems based on novel technologies (1).

Report recommendations

The primary goal of the regulatory science strategy was to ensure that regulation can support the development of new medicines and innovative techniques, including manufacturing processes. This goal required even closer collaboration with academics and research centres to ensure an ongoing dialogue between regulators and developers at all R&D stages (1).

New vaccines are among products requiring new manufacturing processes because of the need for rapid scale-up and fast production to bring them to patients as quickly as possible. These processes may seem to be contrary to the trend towards the development of complex manufacturing systems for the production of personalized advanced therapy medicinal products (ATMPs), which could be made at the point of care. However, there are also similarities. Novel vaccine production processes, particularly those requiring large quantities in response to pandemics, such as COVID-19, need a lot of flexibility and possibly different standards of good manufacturing practice (GMP).

The report recommended that the creation of novel manufacturing technologies should be enhanced by regulators being trained in their assessment. This would help the identification of potential production problems while strengthening the early interaction between regulators, developers, and other stakeholders on the regulatory requirements for innovative manufacturing technologies.

The introduction of new manufacturing processes will probably need, for regulatory purposes, the involvement of EMA's four-year-old Priority Medicines (PRIME) system under which early scientific advice to developers of medicines addressing unmet needs is provided by the agency's experts.

In the regulatory science report, EMA conceded that PRIME is resource intensive, while its purpose is not properly understood by some stakeholders. EMA recommended improved external communication about the scheme and proposed that measures should be taken to shorten PRIME review times. Furthermore, EMA suggested that the system should be reviewed in five years.

The agency also acknowledged that the involvement of Health Technology Assessment organizations (HTAs) in PRIME is crucial so that the scientific advice takes their requirements into account. In their responses during the consultation, HTAs wanted closer alignment with regulators in areas such as pre-approval scientific advice. Whereas, pharma industry respondents were hostile to HTAs being involved in decision making because it complicates and politicises the licensing process, according to the analysis (4).

Divided opinion

This division of opinion was evident in a range of responses to recommendations in the regulatory science report; although on the whole, the recommendations were welcomed by respondents across the five clusters. Ironically the pharma industry and the research sector, whose representatives made up the majority of respondents, were the most supportive of the research science strategy. Among the organizations that tended to be the most critical and sceptical were public bodies.

Some HTAs were against EMA extending its role in areas, such as biosimilars uptake and drug shortages, according to the analysis of the responses (4). In addition to medicines authorizations, the agency should concentrate on information sharing and facilitating co-operation between stakeholders, some HTAs claimed. Payers were concerned that EMA's expanded role in bringing new medicines to market, such as through PRIME, made it a 'co-developer', while it should be restricting itself to the demonstration of clinical benefits and assuring a positive benefit/risk balance. Among EU regulators, national competent authorities (NCAs) for medicines licensing had doubts about how the regulatory science strategy would be turned into action and how NCAs would be involved in its implementation.

Overall, regulators and institutions questioned the sustainability of the EU's current regulatory system in areas, such as updating guidance documents and the effective functioning of working parties and committees (4). No doubt some of these differences with stakeholders will be resolved by the imminent finalization of the EU's regulatory network strategy to 2025 and the publication of the European Commission's own strategy for pharmaceuticals.

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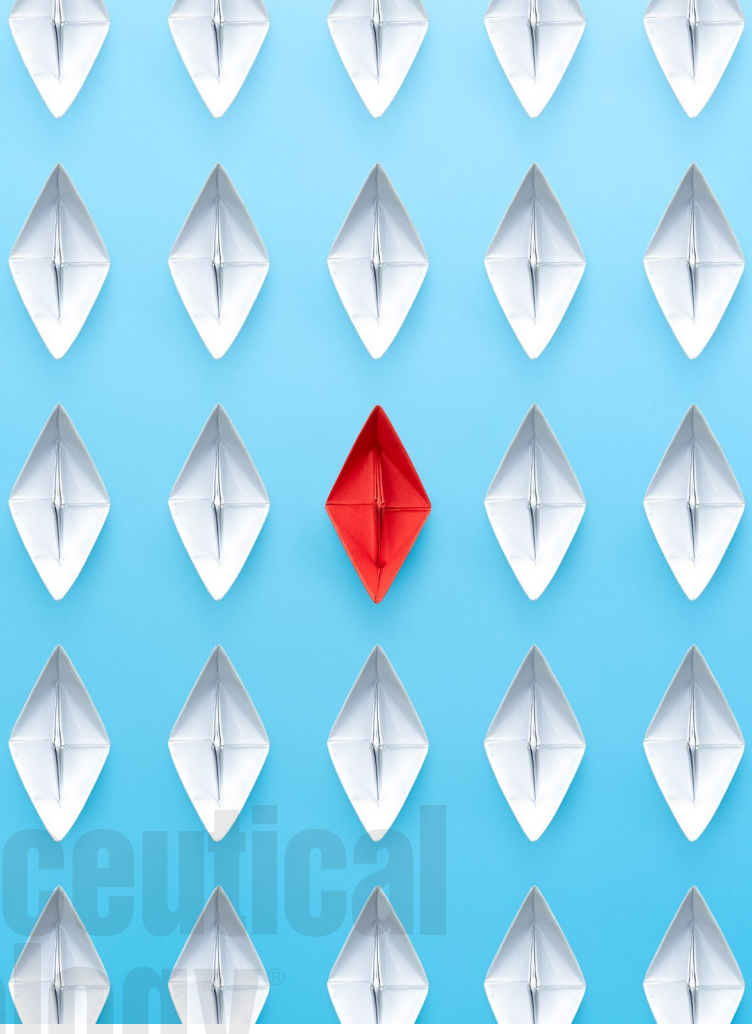

Winds of Innovation Drive Bioprocessing

Technology advances that improve product yield, cut costs, and streamline integration promote smooth sailing for upstream and downstream processes.

Feliza Mirasol

Over the years, many advances have been made in bioprocessing as biomanufacturers strive to increase yield, improve product recovery, enhance product purity, and streamline manufacturing. Innovations in technology and equipment for both upstream and downstream processing have led to more integrated and efficient processes, but there still remain manufacturing challenges that drive the need for further innovation.

Upstream advancements

Successful upstream bioprocessing innovations have focused on key areas, including:

- Increasing volumetric productivity through process intensification (high seed fed-batch, perfusion, and continuous culture technologies)
- Increasing cell-specific productivity and control of critical product quality attributes (CPQAs) via novel cell expression and inducer technologies with greater molecular and cellular biology understanding through systems biology
- Accelerating the overall process development timeline.

"These improvements have resulted in the ability to quickly advance from cell-line generation to clinical current good manufacturing practices (cGMP) manufacturing with bioprocesses that are more productive and reproducible," observes Brian Follstad, director, upstream process development, Catalent Biologics.

"There have certainly been a number of revolutionizing, innovative approaches in bioprocessing in different product areas," says Vasily Medvedev, process development manager at Univercells. "If we consider monoclonal antibody (mAb) manufacture, one of the key bioprocessing advances seen over the years relates to the use of high cell density prefusion cell cultures. This technology reduces the size of the bioreactor needed, directly influencing the overall footprint of operations, capital expenditure (CAPEX), and, subsequently, cost of goods."

Furthermore, Medvedev says the use of design of experiment (DoE) techniques is a promising trend in the industry, when these principles are correctly applied to process development and scale-up studies. "In order to fully benefit from the use of DoE studies, representative scale-down models are paramount to ensure identification of high-performing small-scale process design with seamless transfer to commercial manufacturing scale, reducing development timelines (time to market), and costs."

Looking at the past five years in upstream bioprocessing, Thibaud Stoll, global head of operations, biologics, at Lonza Pharma & Biotech, notes three

main innovations that have impacted bioprocessing operations:

- Continuous improvements in cell expression systems, leading to processes with increasing titers, reaching or even increasingly exceeding the 10 g/L-level
- Continuous development and improvement of disposable bioreactors and associated equipment
- Development of digital tools as part of the digital transformation of biomanufacturing.

"These innovations have contributed to improving process robustness in general, lowering cost of goods and services and enhancing flexibility to respond to fast-evolving demand," Stoll states.

Other innovations, such as media development, cell line selection, improvements to host cell lines, optimized vectors, and the introduction of high-throughput screening technologies, have also revolutionized upstream bioprocessing, adds Atul Mohindra, senior director, research and development, Lonza Pharma & Biotech. Mohindra also counts improved process modelling tools, which have enabled a better understanding of the cell culture process as well as the development of more advanced technical equipment (e.g., single-use technologies [SUT], in-line testing technologies) as important upstream innovations. "This has enabled the industry to develop more complex molecules, to significantly shorten the time taken to manufacture a first-in-human batch as well as to reduce the costs of a development programme," Mohindra says.

"Besides the latest generation of single-use stirred tank and rocking motion (RM) bioreactors, one of the most significant recent introductions has been alternating tangential flow (ATF) filtration," remarks Gerben Zijlstra, global technology consultant, continuous and intensified biomanufacturing, Sartorius Stedim Biotech, who notes that ATF provides for much more efficient and cost-effective perfusion culture than previous methods. "It is also gentler on cells, resulting in higher cell viability and lower levels of impurities to process downstream," Zijlstra adds.

"This technology has enabled tremendous intensification of mammalian cell cultures by allowing 5–10-fold higher cell densities compared to traditional fed-batch processing," Zijlstra explains. By applying different ATF filter pore sizes, continuous upstream manufacturing can be performed as either a dynamic or continuous perfusion, where the product passes the filter and is direct captured using continuous chromatography; or it can be performed as concentrated fed batch (CFB), where the product is retained in the bioreactor and is harvested batchwise, he says. "The productivity of these intensified cell culture processes greatly surpasses those of existing fed-batch platforms. For CFB, titers of around 30 g/L have been reported, while for dynamic perfusions, 60 g/L (equivalent) titers were reported," Zijlstra says.

Another recent innovation is the introduction of several online process analytical technology (PAT) tools, such as cell density monitors that use capacitance sensors, adds Thomas Erdenberger, also a global technology consultant, continuous and intensified biomanufacturing, at Sartorius Stedim Biotech. "These sensors allow real-time cell density monitoring of viable cells without having to measure density using traditional methods by taking a sample, risking contaminating the culture. This new method allows the automation of bioreactor feeding as well as cell bleeding using the sensor coupled with a supervisory control system. At Sartorius, we can place these monitors in any of our single-use bioreactors and RM bioreactors to fully automate the entire seed train and main bioreactor," Erdenberger says.

Another PAT tool gaining traction is Raman spectroscopy. Previously, the key difficulty of using this technology was the need to "train" the mathematical models, the correlation of individual metabolites with the complex Raman spectra. "To solve this challenge, Sartorius recently launched a scalable Raman probe interface so these models are already preset in the 15-mL ambr [Sartorius] high-throughput mini bioreactors. This allows deep process insight and improved process understanding from early development, onwards," states Erdenberger.

Meanwhile, the latest generation of depth filters has also been a powerful innovation for upstream bioprocessing, emphasizes Peter Levison, executive director of business development at Pall Biotech. "As bioprocessing trends have continued to evolve, SUT have offered an alternative solution to drug manufacturers to accommodate shifting drug profiles. Yet, this presented a new challenge when looking at the clarification step," according to Levison.

In traditional stainless-steel facilities, centrifugation has been a widely adopted solution for clarification, but when working in smaller facilities that deliver higher cell densities, such as the newer SUT installations, centrifugation is no longer as feasible, Levison explains. "Not only is it costly to implement, requiring large capital and process investments, it also has a larger footprint and does not scale down so easily. So, while centrifugation is well suited for 10,000-L stainless steel bioreactors and other large facilities, it is not an ideal solution for facilities based around 2000-L single-use bioreactors that many manufacturers use today," Levison asserts.

Initially, depth filters offered an alternative to centrifugation with some performance limitations—traditionally handling cell densities of up to around 20 million cells/mL. "With advances in cell culture and titer increases, we are routinely pushing cell densities up towards the 30 million cells/mL mark, and this is where advanced depth filters deliver the next generation of clarification. The high-performance platform is flexible to support semi- to fully continuous bioprocessing, allowing users to process more product per unit of bioreactor volume. To achieve this performance improvement, two dual-layered depth filtration stages are combined into one clarification step with a flexible chassis to accommodate the capsule configuration needed to

deliver consistent filtrate quality in a significantly decreased footprint," Levison states.

For gene therapy products, the traditional technologies for cell culture and virus production are not suited for commercial-scale manufacture, notes Tania Pereira Chilima, deputy technology officer at Univercells Technologies. "This is not only due to capacity constraints, as these flasks are only compatible with a scale-out approach, increasing costs, and CAPEX, but also due to the labourious nature of operations associated with these technologies and the lack of control over critical process parameters (e.g., pH, dissolved oxygen). This poses some regulatory concerns related to process reliability and reproducibility. Moreover, there is an industry-wide shortage of skilled labour, which means that labour-intensive processes are not as feasible due to resource constraints," Chilima states.

The use of bioreactors for viral vector manufacture poses several benefits, making cell culture and virus production possible in a highly controlled microenvironment. Moreover, these systems are highly scalable and can benefit from the incorporation of PAT to increase the level of process control while simplifying operations. The most advanced bioreactors for cell culture and virus production incorporate principles of process intensification to enable high-titer and low-footprint virus production, Chilima says.

"Technology improvements in process intensification and connection of unit operations have enabled manufacturing updates to run more efficiently and produce higher yields in less time and space, reducing the amount of capital investment," concurs Darren Verlenden, head of bioprocessing, MilliporeSigma. "In upstream, we've seen that utilizing perfusion technology can increase cost efficiencies, decrease risk, and enhance manufacturing flexibility. Between 50–60% of companies are already exploring or have implemented perfusion technologies for seed train or production bioreactor steps," he explains.

Downstream improvements

Innovations in technology and equipment have also benefitted downstream bioprocessing. Recent innovations, which include acoustophoresis (ultrasonics) cell separation and high-precision microfluidics for label-free cell selection, in-line cell washing, and rapid gene delivery, have resulted in significant productivity gains, states Jenna Balestrini, head of precision medicine and cell bioprocessing at Draper, a Cambridge, MA-based not-for-profit engineering firm.

To accomplish cell separation on a clinical blood sample, for example, Draper developed a system that performs acoustophoresis in a high-performance microfluidic device compatible with a range of patient materials and input volumes. This system bypasses the need for centrifugation. "The module continuously and rapidly removes interfering cell contaminants without compromising cell health. With less handling than conventional approaches, acoustophoresis improves end-to-end yield of cells and accelerates delivery to downstream steps in the process," according to Balestrini.

In the gene therapy space, Draper has developed a microfluidic transduction module that can co-localize viral vector around cells, increasing viral-cell interaction while using about half the viral vector typically needed to achieve high transduction efficiency. This allows for more controlled viral gene delivery. The system can transduce at standard efficiency levels in 90 min using a wide range of vector sources, Balestrini says.

And finally, to allow for a variety of payloads, such as ribonucleoprotein, mRNA, or DNA to be introduced into the cell without the need for viral vectors or even activation steps, Draper has engineered a practical continuous-flow electroporation module and in-line buffer exchanger that uses high-precision microfluidics to tightly control cells' exposure to electrical signal, increase throughput, reduce manual-touch labour, and allow for in-line wash steps, Balestrini explains.

"Groundbreaking innovation is seen across different product classes in downstream processing," adds Medvedev. "In mAb manufacture, the advent of continuous purification processes—namely, chromatography—has enabled significant improvements in process productivities, yields, and utilization of key materials (e.g., protein A resin)."

Chilima further adds that in the gene therapy field, the use of alternative media (e.g., monolithic or membrane chromatography, as opposed to traditional bead-based separation) has proven to increase the dynamic binding capacity (DBC) and reduce processing times as this alternative media can be operated at higher flowrates. "Moreover, the use of membrane and monolithic chromatography systems with advanced separation modalities has enabled a consistent increase of resolution in the separation of empty and full capsids to be achieved with this type of media, which is critical in gene therapy manufacture," Chilima says.

The innovations in downstream processing have had similar impacts as in upstream processing innovations, namely the development of continuous manufacturing—in particular, continuous chromatography—that can increase facility throughput while reducing costs and the further development of disposable equipment and digital tools, Stoll says.

"Improved *in-silico* and *in-vitro* modelling tools, which, when combined with high-throughput screening technologies, can increase our capabilities and reduce time," says Mohindra. Further downstream processing innovations he identifies are the introduction of end-to-end, single-use solutions for clinical manufacturing (e.g., prepacked columns, single-use flow paths) and the development of new, high-binding capacity resins.

Erdenberger further highlights the development of newer resins as a particularly beneficial innovation. "Firstly, for the capture step, improved (Protein A) resins with higher binding capacity and improved caustic compatibility

have allowed for substantially improved downstream processing productivity, reduced cost of goods, and improved bioburden control," Erdenberger observes. "Currently, even 'closed, sterile' chromatography seems to be within reach with gamma-irradiated pre-packed columns, enabling continuous, no/low bioburden chromatography operations. In parallel, continuous downstream technologies such as multi-column and simulated moving-bed chromatography have matured and are increasingly implemented to intensify downstream processing," Erdenberger adds.

For the polishing steps, substantial improvements in mixed mode resins, membrane adsorbers, and associated equipment are now, and increasingly, allowing for flow-through polishing as a highly efficient mode of operation, Erdenberger states. He further explains that, for the virus removal steps, methods that allow for continuous virus inactivation and virus filtration are being introduced and that continuous methodologies are even available for cross-flow filtration.

"These technologies result in continuous or semi-continuous product streams to the next unit operation in a process and in much more efficient chromatography at reduced media cost. Processing is also more rapid. When connected with certain upstream operations such as perfusion, the downstream process can be directly connected for a continuous transition from upstream to downstream," Erdenberger notes.

Integrated efficiencies

These advancements in both upstream and downstream processing has had a mixed impact, but overall a beneficial one. While creating the requirement for more well-thought-out and nuanced processes, they have also allowed for closer integration between upstream and downstream. "Although these innovations have increased bioprocess complexity, they have contributed to substantially reduced overall costs, time, and risk in generating drug substance and product. In some bioprocesses, for example, initiating culture harvest while the production culture is still running over the course of a few days (or weeks in continuous) allows purification to begin earlier when compared to historical approaches. Furthermore, on-line analytics and product attribute control strategies permit the measurement and real-time adjustment of CPQAs during a batch, allowing for a more efficient method of reproducibly producing drug substance," remarks Follstad.

"Continuous improvement and interplay between bioreactor productivity and advancements in downstream unit operations are increasing efficiency and streamlining manufacturing," adds Verlenden. "An initial response to upstream intensification includes single-pass tangential flow filtration for downstream debottlenecking. In new facilities, implementation of continuous capture and flow-through polishing can remove process constraints while enhancing facility fit by reducing buffer requirements by up to 47%," Verlenden states.

Continuous biomanufacturing will play an integral role in better integrating upstream and downstream operations over the next five years as manufacturers look to suppliers for integrated solutions because they are thinking holistically about their processes while visualizing future scale up, Verlenden explains. "Our customers expect that, in five years, 40–50% of their processes will incorporate continuous capture and flow through polishing technologies, though adoption of fully continuous processes from end-to-end is likely to be further out," he estimates.

Hearkening back to traditional batch processes, Levison notes that traditional processes are inherently more disconnected step by step. In the upstream, more effective clarification with advanced depth filtration offers a flexible solution. "Users can work towards a semi- or fully continuous processing approach with continuous streams of feedstock, which also impacts the ability to integrate downstream processes. With the ability to integrate the downstream, manufacturers can create more efficient processes, increasing product quality, saving time and money, and maximizing overall productivity and facility utilization," Levison says.

Another development of note on the cell-therapy biomanufacturing front, meanwhile, is the integration of a complicated multistep process into a closed, modular, automated benchtop system that enables effective, safe biomanufacturing that can be used in a hospital (point of care) or in a central manufacturing facility, says Balestrini. Current instrumentation and methods for manufacturing cellular therapies are expensive, time consuming to use, difficult to scale, and limited in their ability to effectively deliver genetic material, Balestrini notes. "A modular system is emerging as an industry gold standard," she states.

Yet, despite these advances in bioprocessing, additional work is still required to ensure that upstream and downstream processing are smoothly integrated, interjects Medvedev. He points to the struggle that current single-use downstream processing technologies for antibody purification are coping with—namely the increasing titers achieved in upstream processing. In gene therapy, on the other hand, the high performance of membrane, beads, and monolithic chromatography systems has caused a mismatch between what upstream processing is able to deliver and what downstream operations are able to purify.

"This is caused by the fact that current technologies for viral vector purification were adapted from the protein industry, which makes them extremely oversized for viral vector applications. The DBC achieved using the current resins available is high with respect to the product harvested in upstream processing. This may cause manufacturers to pull different batches together with intermediate freeze steps, which has disadvantages in terms of yield loss, cold storage space, batch-to-batch variability concerns, and overall process complexity," Medvedev explains. **PTE**

Formulating an ADC Development Solution

Many antibody-drug conjugates are in the pipeline, but only a handful have been approved. Where are the bottlenecks?

Cynthia A. Challener, PhD, is a contributing editor to *Pharmaceutical Technology Europe*.

The concept of bioconjugation has been recognized for many decades, as has the specific idea of antibody-drug conjugates (ADCs). Technology for the production of ADCs did not advance sufficiently until the late 20th century. Even then, the first ADC approved by the US Food and Drug Administration (FDA)—gemtuzumab ozogamicin (Mylotarg) from Pfizer in 2000—was voluntarily withdrawn in mid-2010 due to its association with the serious liver condition called veno-occlusive disease.

Gemtuzumab ozogamicin was ultimately re-approved in 2017 at a lower recommended dose and schedule in a different patient population. There are now six additional FDA-approved ADCs on the market. And, there are approximately 80 ADC candidates undergoing nearly 600 clinical trials (1). Why have more not already been approved? The issue of toxicity has been a challenge, as has the complexity of ADCs and the consequent manufacturing challenges.

Crafting the right molecule

Finding the right target, protein, linker, and payload all belong to the discovery phase. "This phase is very challenging, as the correct definition of the drug is key to move it into the clinic successfully. ADCs represent a product class that is also highly complex and difficult to predict," comments Iwan Bertholjotti, director of commercial development for bioconjugates with Lonza. It is, in fact, one of the key questions in the development of ADCs since the rules are still being written, as data from clinical trials are used to guide ADC technology improvements, says Steve Coats, global project leader at AstraZeneca.

Finding the right targets and crafting an effective molecule has proven to be much more difficult than many researchers had expected at the outset of ADC research, adds Justin Sweeley, senior technology manager, biologics at Novasep. "It was initially understood that finding a good target antigen for an ADC-based compound would require a target with rapid internalization once it had reached the cell," he notes. "The result," he says, "was that in many cases targets that were ineffective for monoclonal antibody (mAb) therapies because of rapid internalization became perfect targets for ADC therapies."

This picture became much more complex upon the addition of payloads to these mAb candidates, however. This issue can be seen most clearly in the fact that the clinical trial landscape includes well over 50 different payload candidates, but only seven have successfully made it into commercial production, according to Sweeley.

"As a result, there has been a recent shift in the industry to recognize that when trying to predict ADC effectiveness, researchers must take into account the target epitope, the physical attributes of the payload on the mAb once conjugated, and also the effect of the payload mechanism of action on the specific kind of cancer being targeted," Sweeley observes.

AstraZeneca takes an empirical approach to target and payload selection, using data from clinical trial results and non-clinical data to help validate target and payload selection. "With potency being driven by the strength of the warhead, we optimize the therapeutic index of our molecules by selecting the most favourable drug-to-antibody ratio (DAR) in designing our ADCs," Coats says. "We believe," he adds, "that establishing target rationale and strategy early on are key to determining the right payload."

Is site-specific conjugation important?

Site-specific conjugation is looked at as the second generation of ADC conjugation techniques. "The homogeneity of these molecules allows for much tighter control at both the manufacturing and characterization levels," Sweeley explains. Better definition for an ADC means that its therapeutic index can be further improved, adds Bertholjotti.

Additionally, the technology allowing for site-specific conjugation has evolved dramatically from the Thiomab concept piloted by

Genentech to current techniques allowing site-specific conjugation with any native mAb, such as Synaffix's glycogen modification techniques or Ajinomoto's AJI-cap technology, Sweeley remarks. Many of the ADCs in preclinical and clinical stages are based on site-specific conjugation technology of one form or another, Bertholjotti adds.

On the other hand, Sweeley notes that even though more site-selective ADCs are expected in the future, ADCs with stochastic conjugation can still be successful. Coats agrees that site-selective conjugation does not seem to demonstrate significant differences in terms of clinical activity and safety when compared to classic non-site-selective conjugation. In fact, the seven commercially approved ADCs are not based on site-specific conjugation.

It is possible, according to Sweeley, that the lack of a commercially available site-specific conjugated molecule is just a result of the head start that stochastic conjugation has had, but it is also possible that simplicity in manufacturing and homogeneity in analytic testing methods doesn't directly result in better outcomes for patients.

Linker technology matters

Finding an ADC that is better than standard therapy or that provides a solution to an unmet need definitely represents a challenge, just as with other drugs based on different approaches, according to Bertholjotti. The linker chemistry is an important component of ADC and has a significant impact on performance.

"Linker chemistry plays a critical role in *in-vivo* stability, but initially was assumed to have a passive role with the exception of either being stable or labile in the acidic cytosolic cellular environment," observes Sweeley. More recently, however, he notes that there has been growing recognition that the linker plays an active role in conjugate hydrophobicity and therefore stability of the ADC as a whole.

The technology, says Coats, has advanced and matured during the past 20 years to a point where linker technology is stable and molecules in development demonstrate low levels of deconjugation in patients. Protease cleavable and non-cleavable linkers are in development and on the market.

Classic conjugation chemistry approaches include maleimide and N-hydroxysuccinamide-ester moieties (Seattle Genetics, Roche, and Pfizer). There are also various newer technologies for linkers, with some, Bertholjotti comments, at the proof-of-concept stage and others already in preclinical evaluation.

An example pointed out by Sweeley is the use of non-natural amino acid-based click-chemistry (Ambrx and Sutro). "To my knowledge, these technologies have clearly simplified the conjugation process, but have not shown a clear improvement in product effectiveness in the clinic," he states.

Undesired immune responses

ADCs are designed to be more selective than traditional chemotherapy agents. The mAb enables targeting of cancer (or other disease) cells where the payload is delivered with high selectivity, thus reducing the systemic toxicity in comparison with standard chemotherapeutic drugs.

Unfortunately, undesired immune responses have presented a problem that has been difficult to overcome. These responses are largely due to the massive number of variables being examined and the relatively small number of clinical trials going on, according to Sweeley. "Many of the warheads being investigated for ADCs induce immunogenic cell death, which may enhance anti-tumour immunity," adds Coats. He also observes that there are recent examples demonstrating clinical activity with a combination of an ADC with PD-1 inhibition.

"In the process of taking any ADC through the approval process, it is necessary to look at the mAb, the linker, and the payload chemistries individually, the ADC as a whole, and the payload and linker residues after the ADC has been internalized and digested within the cell. The end result is that for each ADC trial, researchers must monitor all of the normal undesired immune responses that occur during any oncology trial, but then try to attribute the cause of any that are observed to one of the five different potential sources," Sweeley explains.

"Factoring these additional considerations into the normal variables of any trial, such as the specific cancer type, patient population, and level of pretreatments, etc., the picture becomes incredibly complex. Even after 20 years of clinical testing, the issue is still not fully understood," he concludes.

Lower-than-expected therapeutic window

The primary issue affecting ADC approvals today, according to Sweeley, is the lower-than-expected therapeutic window of these therapies. "If the therapeutic window of ADCs were as large as people expected when ADCs first came to light, then many more approvals would already be on the market," he asserts. This window has proven to be much more complicated than originally hoped, however, and therefore the clinical impact less significant than originally imagined.

Here again, the complexity of ADCs themselves is the main problem. For instance, Sweeley notes that if an innovator company wants to test a new conjugation method, they might choose a mAb and payload pair that have already shown success in the clinic. Similarly, a mAb company with a new antigen target is likely to use an established conjugation approach (e.g., that for Adcetris) to lower their risk. "The best molecule, however," Sweeley explains, "might be a combination of multiple new technologies that are too risky to investigate for a small company with a limited budget."

Complex supply chain

The supply chain for ADCs is highly complex as well. ADCs are unique in that their manufacture is an

amalgamation of classical small-molecule production and traditional mAb manufacturing with the added complexity of highly potent drug manufacturing. "All three characteristics are complex on their own, and combining them brings the complexity to a level where it is no surprise that the vast majority of ADCs are manufactured by outsourcing partners," Sweeley says.

For the most part, he notes that innovator companies treat the manufacturing of an ADC as three separate processes:

- mAb manufacturing using traditional mAb techniques
- Payload synthesis using traditional highly potent synthesis techniques
- ADC conjugation using specialty contract manufacturing organizations capable of both working with both biologics and highly potent payloads.

"Taken individually, all three of these steps are actually defined quite well and have been orchestrated successfully for a long time. But because ADC manufacturing requires all three to happen together, any delays or issues in one process will necessarily affect the other two. The reality, therefore, is that ADC supply-chain management is one of the most complex processes in the pharmaceutical industry and must be managed by an experienced team who can mitigate risks whenever possible," observes Sweeley.

Bertholjotti agrees. The supply chain for ADCs is complex and requires specialized companies to manage different steps in a safe way and with the necessary quality. "If the supply chain is not properly managed, delays and supply issues may arise. Therefore, compromises related to the supply chain can result in critical impacts on timelines and costs to bring a drug to market," he asserts.

Support from FDA

FDA has shown willingness to work closely with sponsors to ensure that ADCs that truly benefit patients are brought to market expeditiously, says Coats. For example, he comments that AstraZeneca's ADC Enhertu (developed in collaboration with Daiichi Sankyo) demonstrated significant benefit in a high unmet-need cancer population. FDA approved Enhertu four months prior to the FDA goal date for the treatment of adult patients with unresectable or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2 based regimens in the metastatic setting. Enhertu is manufactured using site-selective conjugation and contains a cleavable linker technology that releases a warhead with bystander activity. It has also shown encouraging clinical activity in other HER2-driven cancers.

Prudence and integration

Over the past decade, awareness of these issues has increased dramatically, leading to the development of more optimized molecules. Sweeley emphasizes that the complexity of the space means that it is still prudent to go slowly and only tackle one challenge at a time. "Progress is therefore slow, but continually happening. Each success creates a new platform for companies to reach out a little bit farther. And as the industry matures, the rate of success will increase, just as it has in every other area of pharmaceuticals," he asserts.

Novasep has strong expertise in highly potent compound synthesis and for the past three years has brought this expertise into the realm of ADC conjugation, Sweeley notes. "Our focus has always been on producing the highest quality materials by developing the simplest possible procedures and coupling them with world-class analytics. The end result of these efforts is a simplification of the payload synthesis and conjugation steps along the supply chain, enabling rapid development of the two most complicated parts of the manufacturing of ADCs," he states.

Since 2006, Lonza has been establishing a centre of excellence for the development and manufacture of ADCs. Today the company offers an integrated solution, according to Bertholjotti. Integrated offerings afford ADC developers with better predictability of timelines and costs, along with substantially reduced complexity and supply risk, he says.

AstraZeneca is developing novel ADCs and building a library of payloads using its antibody engineering expertise for site-specific conjugation and next-generation ADCs. "ADCs form one of AstraZeneca's key oncology scientific platforms, along with immuno-oncology, DNA damage response, and tumour drivers and resistance mechanisms. Within these platforms, multiple technology and scientific options offer great potential to yield effective medicines for cancer patients," Coats says.

As more ADCs are approved commercially, Sweeley believes a roadmap will be established for other companies to follow, leading to more success in the near future. "In the last 12 months alone, the number of approved ADCs on the market has more than doubled, and I would expect that this track record will be followed by a sizable increase in the amount of research being put into the molecule discovery and preclinical trial stage," he observes. "With the healthy increase in candidates being brought into the preclinical stage, it is only a matter of time until some of those candidates make it through the rigorous demands of clinical testing and reach the market in the not-so-distant future," Sweeley concludes.

The increased number of ADC candidates in the pipeline in combination with ever improving knowledge and the further development of new conjugation technologies will result in an increased rate of approvals, agrees Bertholjotti. Lonza expects that the number of ADC molecules will in fact rise at a 10% compound annual growth rate until 2029.

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A Welcome Change: The Benefits of Reformulation

Reformulation strategies can provide drug developers with a head start to achieve promising options that benefit the patient.

Felicity Thomas

A recent study has estimated the mean cost of developing a new drug and bringing it to market is US$1.3 billion (1). Given this high investment value, the fact that drug development is fraught with potential failure and the increasing complexities of developing difficult-to-handle novel chemical entities, reformulation strategies can provide developers with promising options.

"Due to the high costs of drug development, as well as the high rate of clinical failures, it's vital that pharmaceutical companies evaluate formulation opportunities for every approved or late-stage clinical product to determine how its commercial lifecycle can be optimized," explains Paul Spencer, head of Pharmaceutical Polymers and Services, Evonik Health Care. "To minimize regulatory risk and accelerate speed to market, it is common for pharma companies to seek initial approval for products with a simple lead formulation followed by the introduction of superior formulations that can expand the patient population or enhance safety and efficacy."

Getting a head start

"Reformulating an existing drug gives developers a head start, rather than developing a whole new drug from scratch," says Jeremy Drummond, senior vice-president of Business Development, MedPharm. "This approach cuts product development times because studies can often bridge to those of previous regulatory submissions in particular with regard to non-clinical data."

"One of the main benefits of reformulating drug products is increasing patient adherence to medicines," adds Henny Zijlstra, director, Commercial Development, Lonza. Pointing to research from the National Institute for Health and Care Excellence (NICE), Zijlstra reveals that up to half of all patients prescribed medicine for long-term conditions do not take the medicines as intended (2). "Drug developers can help make it easier for patients to take medicines by, for example, changing the tablet or capsule size or geometry to make it easier to swallow or making it so a drug does not need to be taken strictly with or without food," she says.

Additionally, beyond patient compliance, reformulation strategies have the potential to boost rates of brand preference, notes Spencer. "Reformulation may even help extend the product's commercial lifecycle through the use of proprietary formulation technologies that can generate outcomes, which are difficult for prospective generic rivals to replicate," he states.

Reformulation strategies

There are numerous reformulation strategies that are currently employed by the bio/pharma industry, with each offering a different way in which the patient experience can be improved from the original formulation. Examples of reformulation approaches include the following.

Modifying the original release profile. Most reformulation strategies that are pursued by developers of oral and parenteral dosage forms are based upon the modification of the original release profile, notes Spencer. "For oral solid dosage forms, functional excipients can be used to reformulate a product into dosage forms, such as multiparticulates and matrix tablets, to improve product performance," he says.

Additionally, the drug release profile can be modified by using formulation technologies, which can help improve targetability and oral bioavailability. An example of a strategy to improve bioavailability is through the use of amorphous solid dispersions (ASDs), adds Deanna Mudie, principal scientist, Bend Research, Lonza.

"Enhancing the bioavailability can reduce the dose and remove the food label requirement that calls for patients to strictly take the product with or without food, which can improve patient safety," explains Mudie. "Food labels may present challenges to patients with trouble swallowing. Additionally, the food

label sometimes creates further challenges for patients who have to take medication without food, causing them to skip meals or eat at inconvenient times."

Other examples of reformulations of oral dosage forms that have been shown to improve patient adherence include minitablets or orally disintegrating tablets (ODTs) that offer improved taste sensation or swallowability for paediatric populations, confirms Spencer.

"Reformulating an existing drug gives developers a head start, rather than developing a whole new drug from scratch."

—Jeremy Drummond, MedPharm

Changing the route of administration. Administering drug products via an alternative route is another reformulation approach that can overcome limitations of the original form. "For example, converting from oral to inhalation delivery may allow developers to mitigate side effects associated with the drug interacting with the gastrointestinal (GI) tract," says Mudie.

"For some small molecules and peptides, it is possible to change the route of administration from oral tablets to injectable microparticles and implants," adds Spencer. "There are a range of successful reformulation examples in this regard, such as a shift from orals to extended release, injectable dosage forms to enhance the treatment of schizophrenia, opioid, and alcohol addition, as well as contraception therapies."

However, careful consideration is required when reformulating oral dose products as topicals, cautions Drummond. "In this circumstance, the route of delivery is significantly different, and formulation composition can have a major impact on efficacy as well as chemical and physical stability," he says.

In-vitro models that are based on relevant human tissues to provide results that are directly relatable to clinical outcomes and can also provide answers to specific research questions can help to optimize reformulations, asserts Drummond. "Using these models often leads to novel discoveries that can extend existing patents, making reformulation an attractive option for drug companies," he states.

Reducing dose frequency. Injectable microparticles that use the biocompatibility and resorption attributes of lactide/glycolide polymers can help to reduce administration frequency or enable localized delivery of drugs, reveals Spencer. "Lactide/glycolide polymers have attained decades of literature in precisely controlling the rate of drug release from microparticles over weeks, months, or a year or more following a single administration," he says.

"Drug developers may also reformulate drug products as controlled-release versions," adds Zijlstra. "This method can assist in reducing the dosing frequency, thus reducing the pill burden on patients."

According to Spencer, ocular drug delivery is currently experiencing intense focus in terms of reformulation. "In ocular drug delivery, it is a therapeutic priority to minimize the number of required intravitreal injections per eye," he explains. "Here, pharmaceutical companies are developing drug-loaded implants that can safely resorb via hydrolysis after months of drug delivery."

Combination products. Comprised of two or more components in a single entity, combination products can enable developers to consistently maintain concentrations of API, minimize adverse effects, and reduce the number of dosing units required, confirms Mudie. However, there is the possibility that the single unit may be too large for the patient to swallow, she warns.

Liposomes. "Liposomes have also played a significant role in the successful reformulation of drug products," says Spencer. "Decades-old cancer drugs reformulated into liposomes and pegylated liposomes are therapeutically efficacious with improved toxicity profiles, better cardiac safety, and less side effects. Antifungal liposomes also show reduced toxicity along with extended-release performance, which results in longer retention times of the drug in tissues."

Additionally, through reformulating pain drugs into long-acting liposome preparations, it is possible to reduce the frequency of epidural injections required, Spencer adds. "In all, several drugs reformulated into liposomes have been strongly preferred to the original dosage forms," he states.

"Reformulation may even help extend the product's commercial lifecycle…"

—Paul Spencer, Evonik Health Care

The target patient profile

"When considering drug reformulation strategies, the target patient profile can play a role in developing the most effective product," asserts Zijlstra. Giving an example, she explains that in the case of geriatric patients, who can have issues swallowing larger tablets, it may be beneficial to reformulate a drug product into a multi-particulate or sprinkle-capsule form, thereby enabling the patient the option to sprinkle the medicine onto food or in water.

"Reformulation strategies can be particularly beneficial to a range of chronic diseases and patient population sub-sets, such as paediatrics or geriatrics," agrees Spencer. "Improving rates of brand acceptance amongst patient subpopulations, such as paediatrics, are also a key focus of reformulation strategies. Here, the goal of reformulation might be to reduce

tablet size, enhance surface coating glossiness, or neutralize bad odours to improve taste and swallowability."

For Drummond, the needs of the patient must be considered just as much as the chemical and physical properties of the drug in question, irrespective of whether it is a reformulation or a new chemical entity. "These factors all feed into the target product profile," he adds. "In the case of topical reformulation, transforming an oral product to a topical formulation can often mean moving from systemic delivery to local delivery, which is important to consider when assessing the target patient group."

"Converting from oral to inhalation delivery may allow developers to mitigate side effects associated with the drug interacting with the gastrointestinal tract."

—Deanna Mudie, Lonza

In terms of specific disease area, prostate cancer patients have been shown to improve compliance after reformulation of daily injections to a single injection, extended-release, dosage form was done, Spencer continues. "Another example is the reformulation of a drug from a daily oral tablet to an extended-release parenteral dosage form that has improved compliance in schizophrenic and bipolar patients," he says. "In addition to eliminating the need to remind patients to take their medication, it has also helped addiction patients from intentionally and prematurely stopping their therapy."

Adding to Spencer's comments, Zijlstra explains that some relief can be offered to oncology patients if the food label can be removed from the medicine through reformulation. "Many oncology patients have reduced appetites as a side effect of chemotherapy," she notes. "However, the medicines they are required to take often have food labels on them, meaning that in some cases, the patients must take the dose with food. Therefore, a reformulation strategy that can remove this 'food' requirement can improve the patient experience."

"Furthermore, formulations that combine local delivery with extended-release can dramatically improve rates of brand acceptability for patients who would otherwise face a series of uncomfortable intravitreal injections or injections into the knee or other joints," Spencer iterates. "Such reformulation options can help to showcase the long-term cost-effectiveness of the drug product and improve reimbursement options."

Potential path forward

According to Mudie, a potential future reformulation strategy could be a digital one. "For example, developers may insert a chip inside a dosage form to alert either the patient, healthcare provider, or both when the medication has successfully been administered," she says. "This innovation could be useful for oncology patients, who may experience anxiety related to keeping track of their medications. It may also be beneficial for treating schizophrenia, by allowing patients, their families, and their doctors to know with confidence that they have taken their medication on time."

Moving forward, Spencer anticipates there will be an increasing number of applications for local delivery. "In this application, drug concentrations in local tissues can be maximized for efficacy while minimizing systemic drug exposure resulting in reduced side effects via placement of the drug product directly into target sites, such as joints, the spine, the eye, infected areas, tumours, or the brain," he notes.

"Furthermore, local delivery can minimize the total amount of dose required," Spencer continues. "As specialized drugs continue to become more highly potent, the goal of reducing injection volumes and extending the period of drug release will become even more important." As an example, Spencer highlights a recent approval of an ocular implant, which has the capability of releasing 10 micrograms of drug over a four-month period (3). "Calculated linearly, that's only 0.00008 milligram of drug per day," he remarks.

"A reformulation strategy that can remove [the] 'food' requirement can improve the patient experience."

—Henny Zijlstra, Lonza

Another approach that may have promise for the future in Mudie's opinion is the creation of a dosage form that has the ability to release in the GI tract non-traditionally. "For example, it may attach to the intestinal wall and release the drug contents for uptake into the bloodstream," she says. "This approach could benefit patients who require frequent injections or have harsh GI environments."

"Finding new molecules that have acceptable therapeutic profiles is not getting any easier," summarizes Drummond. "Fundamentally, all reformulations, current and future, must benefit the patient, whether by providing a new solution to combating an indication or improving compliance and ease-of-use, to ensure there is no compromise to the patient."

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On-Dose Authentication to Safeguard Patients and Uphold Brand Integrity

ON-DEMAND WEBCAST

Register for this free webcast at: http://www.pharmtech.com/pt_p/safeguard

Event Overview

Smart medicines to prevent counterfeiting and diversion of drug products.

Complex supply chains for pharmaceutical products make medicines increasingly vulnerable to counterfeiting and diversion and can lead to significant loss of revenue and reputational damage for the brand owner, as well as health risks and loss of trust for the consumer.

Going beyond the packaging with on-dose taggant solutions has been advised through the US Food and Drug Administration's guidance on the use of physical-chemical identifiers in solid oral dosage forms.

Sign up for this webcast to learn how brand owners can implement smarter medicines with a more patient-centric approach, while protecting their products from external threats and mitigating drug counterfeiting and product diversion.

Key Learning Objectives

- Understand why serialization is not enough to control counterfeiting of pharmaceuticals
- Gain insight into how medicines can be made smarter with on-dose technology to address patient safety
- Discover how simple addition of on-dose technology can better secure the supply chain with a digital lock and instant authentication

Who Should Attend

Attendees should include C-suite level and senior managers from pharmaceutical manufacturers, distributors, and contract research and manufacturing organizations; brand protection, enforcement and security experts; healthcare research organization representatives, especially for patient adherence and safety; intellectual property and trademark investigators and counsels; drug regulatory agencies and customs officials.

For questions or concerns, email mdevia@mjhlifesciences.com.

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Colorcon Inc.

Moderator

Rita Peters
Editorial Director
Pharamceutical Technology

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Determining the Probability of Passing *USP* Content Uniformity and Dissolution (Immediate and Extended) Tests with CuDAL-Excel

Lei Lei and Pramote Cholayudth

The CuDAL-Excel program, based on Microsoft (MS) Excel, has been developed to calculate the *USP* passing probability of content uniformity and dissolution tests for both sampling plan 1 and sampling plan 2 scenarios and for both immediate release and extended release requirements. The users can obtain the passing probability by simply entering the input variables, with wide applications for process validation/verification and batch release. As a user-friendly program, CuDAL-Excel should bring more benefits to the industry practitioners than other existing programs/tools.

A statistical sampling plan is not included within *United States Pharmacopeia* (*USP*) <905> Uniformity of Dosage Units and *USP* <711> Dissolution, which are only intended to determine conformance of a sample taken from routine batches or the pharmaceutical market. Passing *USP* <905> tests once does not provide statistical assurance that a batch will meet the target quality (1), thus testing against *USP* acceptance criteria is insufficient for batch release or process validation purposes. Statistical acceptance criteria with statistically valid sampling plans can better assess the quality of a process.

For in-process sampling of dosage units, the US Food and Drug Administration's (FDA's) current recommendation is to use nested sampling plans—to test replicate samples (more than one, typically three, which should be sampled at closest positions) from each location of the stratified plan (2). In this way, the data can be subjected to variance component analysis, which divides the total variance into "between location" and "within-location" components (2), increasing the evaluation confidence for batch quality. The "between-location" component is the variability across the sampling locations in a blender, or during compression, encapsulation, or filling process, while the "within-location" component is the variability between samples within a given sampling location.

Bergum published a method to calculate a lower bound on the probability of meeting acceptance criteria of multiple stage tests (3), such as the *USP* content uniformity and dissolution tests, and gave examples in a book (4). Later, Bergum wrote a SAS program (CuDAL version 1), which implements his calculation method (5). In 2007, Bergum revised the calculation method (6), and a newer SAS program (CuDAL version 2) was developed and validated according to the revised *USP* 29 test for content uniformity (7). The CuDAL methodology finally became ASTM E2810 and ASTM E2709, which is referenced for establishing acceptance criteria for a stratified sampling plan. Bergum's CuDAL tool is now the standard practice for demonstrating capability to pass *USP* content uniformity and dissolution tests.

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Table I. CuDAL-Excel program sheets.

	Spreadsheet name	Function	Dosage form	Sampling plan
1	Calculate relative standard deviation (RSD) for sampling plan (SP) 1	Calculate the mean, RSD, maximum, and minimum	NA	1
2	Calculate standard deviation (SD) for SP2	Calculates the mean, within-location SD (denoted as SE), between-location SD (denoted as SM), maximum and minimum	NA	2
3	CUSP1	Calculate the passing probability against *USP* <905> Content Uniformity test	NA	1
4	CUSP2	Calculate the passing probability against *USP* <905> Content Uniformity test	NA	2
5	DissSP1	Calculate the passing probability against *USP* <711> Dissolution test	Immediate-release	1
6	DissSP2	Calculate the passing probability against *USP* <711> Dissolution test	Immediate-release	2
7	ExtDissSP1	Calculate the passing probability against *USP* <711> Dissolution test	Extended-release	1
8	ExtDissSP2	Calculate the passing probability against *USP* <711> Dissolution test	Extended-release	2

*RSD, relative standard deviation; SD, standard deviation; NA, not applicable; SP, sampling plan; SE, within-location SD; SM, between-location SD; *USP*, *United States Pharmacopeia*; CUSP1, content uniformity sampling plan 1; CUSP2, content uniformity sampling plan 2; DissSP1, dissolution sampling plan 1; DissSP2, dissolution sampling plan 2; ExtDissSP1, extended release dissolution sampling plan 1; ExtDissSP2, extended release dissolution sampling plan 2

However, the requirement of SAS software limits the wider use of CuDAL by industry practitioners. Alternative programs based on readily accessible software would be more acceptable. R language programs, transformed from SAS programs, were written by *www.pharmstat.com*. Pramote Cholayudth, coauthor of this paper, first developed (8) and later revised (9) a Microsoft (MS) Excel program to compute the probability of passing a *USP* content uniformity test; however, it is no longer consistent with Bergum's current CuDAL method. The International Society for Pharmaceutical Engineering (ISPE) has also published five common acceptance limit tables for sampling plans 1 and 2 based on ASTM E2709/E2810 and an Excel Workbook (10). However, due to the limited number of combinations of acceptable probability, number of samples and sample locations, and confidence levels, the tables are not capable of satisfying diverse company requirements.

The present work reports the CuDAL-Excel, a set of MS Excel programs transformed and extended from Bergum's CuDAL version 2 SAS program. The CuDAL-Excel is used to evaluate data against the *USP* 29–43 <905> Content Uniformity and <711> Dissolution for both immediate-release and extended-release dosage forms (the test for extended-release dissolution is an additional function to CuDAL). It is designed for industry practitioners for batch release and process validation.

CuDAL-Excel program

The CuDAL-Excel is an MS Excel file that contains eight MS Excel sheets with functions listed in **Table I**, and it calculates the passing probability of result data against *USP* multiple stage tests, <905> Uniformity of Dosage Units and <711> Dissolution. The CuDAL-Excel is based on Bergum's methodology and is transformed and extended from CuDAL SAS programs as well as from R programs written by *www.pharmstat.com*. The calculation requires estimation of statistical lower and/or upper bounds for the mean and relative standard deviation (RSD)/standard deviation (SD) of the samples of appropriate size. The calculation method assumes that the test results can be approximated by a normal distribution. For detailed calculation methodology, interested readers can unhide the hidden MS Excel columns and rows to understand the calculation algorithm. The open-source CuDAL-Excel file is available from the author.

CuDAL-Excel inputs

Overall mean. The calculation of overall mean for both sampling plan (SP) 1 and SP2 are the same. Overall mean = AVERAGE(D2:D101) or = AVERAGE(F3:Y72), where D2:D101 or F3:Y72 are the individual results of all samples. All measurements of dosage units and criteria values are in percentage label claims (%LC).

Calculation of RSD for SP1. RSD = 100 * SD/Mean = 100 * STDEV(D2:D101)/AVERAGE (D2:D101), where D2:D101 are the individual results of samples from all the locations.

Calculation of SD for SP2. Within-location SD (denoted as SE) = SQRT(AVERAGE(D3:D72)), where D3 = VAR(F3:Y3), with F3:Y3 as the individual results of samples from the first location, and D72 = VAR(F72:Y72), with F72:Y72 as the individual results of samples from the last location.

Between-location SD (denoted as SM) = STDEV(C3:C72), where C3 = AVERAGE(F3:Y3), with F3:Y3 as the individual results of samples from the first location, and C72 = AVERAGE(F72:Y72), with F72:Y72 as the individual results of samples from the last location.

Sample size. The sample sizes are different for SP1 and SP2.

- For SP1, one (n=1) dosage unit is sampled from each of the L locations, with a total sample size of L. The locations should be equally spaced throughout the batch.
- For SP2, more than one (n>1) dosage units are sampled from each of the L locations, with a total sample size of n×L. The locations should be equally spaced throughout the batch, while the n samples within a location should be sampled as close as possible.

Confidence level. Confidence levels as well as probability values (P-values) are typically 50%, 90%, or 95%. A Parenteral Drug Association (PDA) Technical Report suggests the use of a 90% confidence level to provide 95% coverage (6). In the regulatory arena, a confidence level of 95% is often used (1).

Probability lower bound (passing probability). The probability (or coverage) lower bound, denoted as "passing probability," is the lowest probability of passing the *USP* acceptance criteria, and 90%, 95%, and 99% are commonly used, with 95% as the usual value employed in practice (1).

Probability of passing *USP* 29–43 <905> Uniformity of Dosage Units test

The *USP* <905> Uniformity of Dosage Units test procedure and acceptance criteria are listed in **Table II** (11). The two-stage test and its acceptance criteria have not changed from *USP* 29 to the current *USP* 43.

Sheet CUSP1 (CU sampling plan 1). The Excel sheet CUSP1 needs users to input cells B1–B5 with the target content, confidence level, number of samples tested, average, and RSD of all sample results. Then cell B6 will display the calculated passing probability. The merged cell range A7–B11 gives the narrative conclusion.

The calculation formulas are demonstrated in the CuDAL-Excel file. With given inputs (cells B1:B5), the statistical lower bound, LLU (B23), and upper bound, ULU (B43), of the mean, can be calculated. For LLU, the passing probability of stage 1 is calculated in B30 and the passing probability of stage 2 is in B41. B28 and B29 are the probability integration results for stage 1, while B35 and B36 are the probability integration results for stage 2. The integration calculation results, int2 (H304, sum of H4:H303), int3 (N304, sum of N4:N303), iint2 (T304, sum of T4:T303), iint3 (Z304, sum of Z4:Z303), and the detailed calculation steps are demonstrated in the CuDAL-Excel file. The maximum one of the passing probabilities of stage 1 and stage 2 (B30 and B41, respectively) is the overall passing probability for LLU (B42). The calculation of overall passing probability for ULU (B62) is the same. The minimum one of overall passing probabilities for LLU (B42) and ULU (B62) is the final passing probability and shown in B6.

For example, if target content = 100, confidence level = 95%, number of samples tested = 30, mean of tested results = 99.9% label claim, RSD of the tested results = 4, the calculated probability of passing *USP* Content Uniformity test for a future sample taken from the batch is approximately 97.78% (0.977799163). The obtained probability is larger than 95%, thus the batch is well within the release requirement, or the process is valid to produce products meeting *USP* Content Uniformity test requirements.

Table II. *United States Pharmacopeia* (*USP*) 29–43 <905> Uniformity of Dosage Units test procedure and acceptance criteria.

Stage (S)	Number tested	Pass stage if:
S1	10	$AV = \lvert M - \bar{X}\rvert + 2.4\,s \leqslant 15.0$, where M is defined below.
S2	20	(1) $AV = \lvert M - \bar{X}\rvert + 2.0\,s \leqslant 15.0$, using all 30 results (S1 + S2). (2) No dosage unit is outside the maximum allowed range of 0.75 × M to 1.25 × M.
M is defined as follows:		
If T ⩽ 101.5 %LC	If $98.5 \leqslant \bar{X} \leqslant 101.5$, then	$M = \bar{X}$ ($AV = ks$)
	If $\bar{X} < 98.5$, then	$M = 98.5\%$ ($AV = 98.5 - \bar{X} + ks$)
	If $\bar{X} > 101.5$	$M = 101.5$ ($AV = \bar{X} - 101.5 + ks$)
If T > 101.5 %LC	If $98.5 \leqslant \bar{X} \leqslant$ T, then	$AV = ks$
	If $\bar{X} < 98.5$, then	$AV = 98.5 - \bar{X} + ks$
	If $\bar{X} >$ T, then	$AV = \bar{X} - T + ks$

Note: At each stage calculate the sample average, $\bar{X}$, and the sample standard deviation, s.
S, stage; AV, acceptance value; s, sample standard deviation; $\bar{X}$, sample average; T, target content; M, reference value; k, acceptability constant.
The information is derived from *USP* General Chapter <905> "Uniformity of Dosage Units" (11).

For another example (as shown in **Figure 1**), if the given target = 100, confidence level = 90%, and number of samples = 18, the passing probability is calculated by CuDAL-Excel with varying mean and RSD. The obtained contour chart shows that passing probability increases with decreasing RSD and with a mean closer to centre target, and that a higher RSD can be tolerated when the mean is close to the centre target.

Sheet CUSP2 (CU sampling plan 2). Sheet CUSP2, compared with sheet CUSP1, has more intermediate parameters (B17–B31 in sheet CUSP2) than SP1 (B13–B15 in sheet CUSP1), due to the more complicated calculation algorithm of SIGMA. These calculations involve the number of samples per location, number of locations, within-location SD, and between-location SD. However, all the other calculation steps are almost the same as sheet CUSP1. The detailed calculation steps are shown in CuDAL-Excel file sheet CUSP2.

For example, if the target content = 100, confidence level = 95%, number of samples tested per location = 3, number of locations = 20, mean of tested results = 99.9% label claim, within-location SD of the tested results = 4, between-location SD = 3.3, the calculated probability of passing *USP* Content Uniformity test for a future sample taken from the batch is about 86.09% (0.860912446). The obtained probability 86.09% is too small to accept, thus the batch is not released or the process needs to be improved.

Figure 1: The contour chart of passing probability against *United States Pharmacopeia* content uniformity test calculated with CuDAL-Excel (sheet CUSP1 of the MS Excel file) from varying relative standard deviation (RSD), based on target = 100, confidence level = 90% and number of samples = 18.

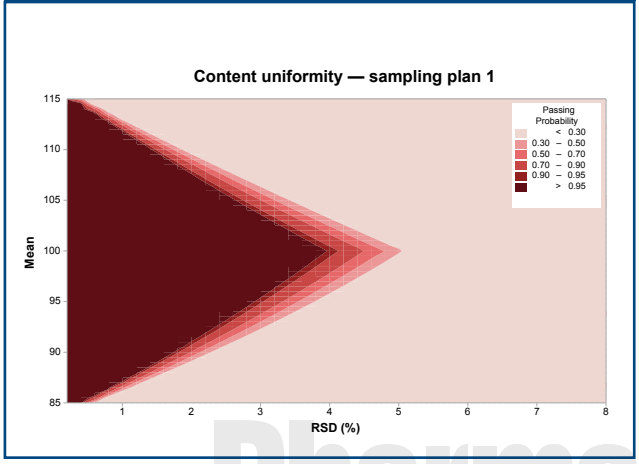
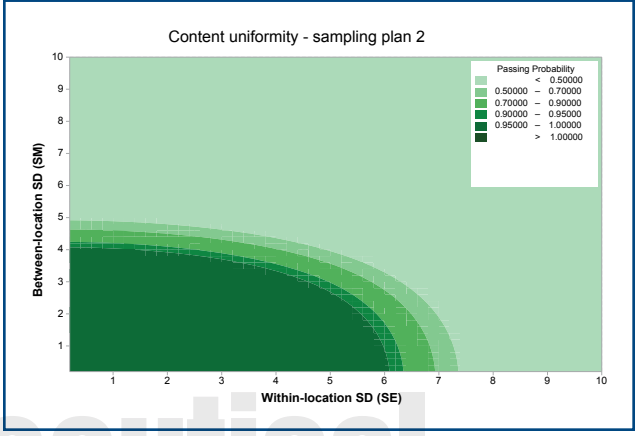


Figure 2: The contour chart of passing probability against *United States Pharmacopeia* content uniformity test calculated with CuDAL-Excel (sheet CUSP2) from varying within-location SD (SE) and between-location SD (SM), based on target = 100, mean = 100, confidence level = 90%, number of samples per location = 3, and number of locations = 20. SD is standard deviation.



For another example (as shown in **Figure 2**), if the given target = 100, mean = 100, confidence level = 90%, number of samples per location = 3, and number of locations = 20, the passing probability is calculated by CuDAL-Excel with varying within-location SD and between-location SD. The contour chart shows that passing probability increases with decreasing within-location and between-location SD. To meet a passing probability, the allowable within-location SD is larger than between-location SD.

Probability of passing *USP* 43 <711> Dissolution test

The *USP* Dissolution test procedures and acceptance criteria are summarized in **Table III** and **Table IV** (12).

Immediate-release dosage form. For immediate-release dosage forms, examples for calculating passing probability of sampling plan 1 and sampling plan 2 are respectively demonstrated below.

Immediate-release SP1 (sheet DissSP1). For example, if the Q limit = 80, confidence level = 95%, number of samples tested =30, mean of tested results = 84% label claim, RSD of the tested results = 5.6%, the calculated probability of passing *USP* Content Uniformity test for a future sample taken from the batch is about 91.16% (0.911628045). The obtained probability 91.16% is acceptable (if there is a predefined acceptance limit of 90%), thus the batch is meeting the release requirement, or the process is valid to produce products meeting *USP* Dissolution test requirements.

Immediate-release SP2 (sheet DissSP2). For example, if the Q limit = 80, confidence level = 95%, number of samples tested per location = 3, number of locations = 20, mean of tested results = 88% label claim, within-location SD of the tested results = 7.5, between-location SD = 5, the calculated probability of passing *USP* Content Uniformity test for a future sample taken from the batch is about 94.31% (0.94309498). The obtained probability 94.31% is acceptable (if there is a predefined acceptance limit of 90%), thus the batch is meeting the release requirement, or the process is valid to produce products meeting *USP* Dissolution test requirements.

Extended-release dosage form. For extended-release dosage forms, examples for calculating passing probability of sampling plan 1 and sampling plan 2 are respectively demonstrated below.

Extended-release SP1 (sheet DissSP1). For example, if the dissolution lower limit = 48%, dissolution upper limit = 72%, confidence level = 95%, number of samples tested =18, mean of tested results = 56%, SD of the tested results = 5, the calculated probability of passing *USP* dissolution test (extended-release) for a future sample taken from the batch is approximately 94.37% (0.943742397). The obtained probability 94.37% is acceptable (if there is a predefined acceptance limit of 90%), thus the batch is meeting the release requirement, or the process is valid to produce products meeting *USP* Dissolution test requirements.

Extended-release SP2 (sheet ExtDissSP2). For example, if the dissolution lower limit = 48%, dissolution upper limit = 72%, confidence level = 95%, number of samples tested per location = 3, number of locations = 20, mean of tested results = 56%, within-location SD of the tested results = 5.2, between-location SD of the tested results = 4.5, the calculated probability of passing *USP* Dissolution test (extended-release) for a future sample taken from the batch is approximately 93.38% (0.933772184). The obtained probability 93.38% is not acceptable (if there is a predefined acceptance limit of 95%), thus the batch is not meeting the release requirement, or the process needs to be improved.

In another example where an extended-release tablet dissolution is tested at three time points, the dissolution specification is when the dissolution percentage at the first time-point is not more than 40%, the dissolution percentage at the second time-point is between 50% and 75%, and the dissolution percentage at the last time point is not less than 90%. When the given confidence level = 95%, number of samples tested per location = 3, and number of

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Table III. *United States Pharmacopeia (USP)* 43 <711> Dissolution test procedure and acceptance criteria, immediate-release dosage forms.

Stage	Number tested	Acceptance criteria
Stage 1	6	Each of the 6 individual units should be equal or larger than *Q* +5%.
Stage 2	6	The mean value of all the 12 units from both Stage 1 and Stage 2 should not be less than *Q*, and none of the 12 units should be less than *Q* – 15%.
Stage 3	12	The mean value of all the 24 units from all three stages (Stage 1, Stage 2, and Stage 3) should not be less than *Q*, the number of units that are less than *Q* – 15% should not be more than 2, and each of the individual 24 units should be ⩾ *Q* – 25%.

Q, quantity, expressed as a percentage of the labelled content.
The information is derived from *USP* General Chapter <711> "Dissolution" (12).

Table IV. *United States Pharmacopeia (USP)* 43 <711> Dissolution test procedure and acceptance criteria, extended-release dosage forms.

Level	Number tested	Acceptance criteria
Level 1	6	Each of the individual values should be within each of the specified ranges, and each of the individual values at the final test time point should be ⩾ the specified amount.
Level 2	6	The mean value of all the 12 units from both Level 1 and Level 2 should be within each of the specified range and the mean value of the final test time point should be ⩾ the specified amount; each of the individual values should be within 10% of label content outside each of the specified ranges; and each of the individual values of the final test time point should be ⩾10% of the labelled content below the specified amount.
Level 3	12	The mean value of all the 24 units from all the three levels (Level 1, Level 2, and Level 3) should be within each of the specified ranges and the mean value of the final test time point should be ⩾ the specified amount; the number of units that are >10% of labeled content outside each of the specified ranges should not be more than 2; the number of units of the final test time point that are >10% of labelled content below the specified range should not be more than 2; and each of the 24 individual units should be within 20% of labelled content outside each of the specified ranges while for the final test time point all values should be equal or larger than 20% of labelled content below the specified amount.

The information is derived from *USP* General Chapter <711> "Dissolution" (12).

locations = 20, the passing probability of data obtained at the first time point is calculated by ExtDissSP2 to be 0.993062574 (inputs dissolution lower limit = 0, dissolution upper limit = 40, mean = 35, within-location SD = 3.4, and between-location SD = 2.9), that of the second time point is 0.995913738 (inputs dissolution lower limit = 50, dissolution upper limit = 75, mean = 63, within-location SD = 4.3, and between-location SD = 4.7), and that of the last time point is 0.930491472 (inputs dissolution lower limit = 90, dissolution upper limit = 1000 [enter a large enough number], mean = 95, within-location SD = 2.9, and between-location SD = 3.7). The overall passing probability is calculated, by manually multiplying the above three probability values, to be 0.920260402 (= 0.993062574 × 0.995913738 × 0.930491472). That means the passing probability of the data tested against product specification as per *USP* procedures is 92.03%.

Validation of CuDAL-Excel

The CuDAL-Excel was validated by comparison of results obtained from the CuDAL-Excel with those from Bergum's CuDAL SAS programs. The results, as listed in **Table V**, show that the above two programs generate the same passing probability values. The calculated intermediate parameters (not shown) are also the same for the two programs. The above results demonstrate that the two programs are equivalent.

As Bergum's CuDAL programs do not cover probability determination for dissolution of extended-release dosage forms, it was not possible to viably compare against the original SAS programs. The authors have done their best to check the calculation steps/algorithm of sheets ExtDissSP1 and ExtDissSP2 under the principle of CuDAL statistical methodology. Users may decide to perform additional validation.

Conclusion

The developed CuDAL-Excel is used to calculate the passing probability of collected data against *USP* multi-stage Content Uniformity and Dissolution tests. The programs are based on Bergum's methodology, and the functions have been validated. Therefore, the CuDAL-Excel can be used as an alternative of CuDAL SAS programs. It is essential that users validate the spreadsheet before use in a regulated environment. The CuDAL-Excel file and its user manual can be freely downloaded from CuDal_Excel@163.com. With this file, the users can obtain the passing probability by simply entering the input values. The CuDAL-Excel can be used for process validation/verification and batch release. As a user-friendly Excel-based program, CuDAL-Excel should bring more benefits to practitioners than the other tools.

Access to tool. The tool can be downloaded from CuDal_Excel@163.com.

Editor's Note: The email address and tool are provided by the authors, and *Pharmaceutical Technology Europe* does not assume any liability for the contents of the linked file.

Acknowledgement

This work fully respects and benefits from James Bergum's great statistical methodology and original SAS programs. The authors' intention is to make the great methodology performable on a software MS Excel which is widely accessible to industry practitioners. The authors are grateful to the reviewers of this paper for their valuable comments.

Table V. Comparison of results from CuDAL-Excel and Bergum's CuDAL SAS programs. LC is label claims.

Content uniformity—sampling plan 1

Target (%LC)	Confidence level (%)	Number of samples	Mean (%LC)	RSD (%)	Passing probability	
					CuDAL-Excel	Bergum's CuDAL SAS
100	95	30	99.9	4.0	0.977799163	0.977799163
104	90	20	98.0	3.0	0.999434038	0.9994340384
98	90	25	101.0	3.8	0.98353442	0.9835344205

Content uniformity—sampling plan 2

Target (%LC)	Confidence level (50.0–99.0%)	Number of samples per location (NN)	Number of locations (LOC)	MEAN (%LC)	Within-location standard deviation (SE)	Between-location standard deviation (SM)	Passing probability	
							CuDAL-Excel	Bergum's CuDAL SAS
100	95	3	20	99.9	4.5	3.1	0.845161545	0.8451615445
104	90	3	20	98	5.1	2.7	0.657672831	0.6576728309
98	90	4	20	98	4.3	2.9	0.818066305	0.8180663055

Dissolution-immediate release—sampling plan 1

Q (%LC)	Confidence level (%)	Number of samples	Mean (%LC)	Relative standard deviation (%)	Passing probability	
					CuDAL-Excel	Bergum's CuDAL SAS
80	95	20	88	7.5	0.921373945	0.9213739454
75	95	15	85	6.9	0.981648546	0.9816485461
80	90	30	84	5.6	0.964116094	0.9641160937

Content uniformity—sampling plan 2

Q (%LC)	Confidence level (50.0–99.0%)	Number of samples per location (NN)	Number of locations (LOC)	MEAN (%LC)	Within-location standard deviation (SE)	Between-location standard deviation (SM)	Passing probability	
							CuDAL-Excel	Bergum's CuDAL SAS
80	95	3	20	88	7.5	5	0.94309498	0.9430949797
80	95	4	25	83	5.6	3	0.83860103	0.8386010299
85	95	3	20	88	3.7	3.4	0.754481816	0.7544818161

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Manufacturing Microneedle Array Patches for Vaccine Delivery

Equipment and process optimization must be considered for scaling up these developmental technologies to commercial production.

Jennifer Markarian

Microneedle array patches (MAPs) have been in development as an alternative to injections for delivering vaccines and other drugs. Microneedle patches differ from transdermal patches that deliver medicine through the outermost layer of the skin (the stratum corneum), because the microneedles pierce the stratum corneum and deliver the drug into the epidermis or upper part of the dermis, but not deep enough to cause pain. Microneedle technologies include solid microneedles coated with the drug, hollow microneedles filled with a liquid drug, and dissolvable microneedles with the drug embedded in a soluble material.

Microneedle patches have been studied in a clinical trial for delivering a flu vaccine (1) and in preclinical trials for delivering inactivated rotavirus vaccine (IRV) and co-administration of IRV and inactivated poliovirus vaccine (2). Recently, MAPs are being investigated for a vaccine to fight COVID-19 (3). The University of Pittsburgh Medical Center (UPMC) announced in an 2 April 2020 press release that its scientists had developed a potential vaccine against SARS-CoV-2 that would be delivered through a MAP (3). The fingertip-sized patch uses 400 microneedles that deliver the spike protein pieces into the skin, where the needles, which are made of sugar and the protein pieces, dissolve. Noting that scalability is crucial for vaccines intended for protection from pandemics, UPMC said that the process to make and purify the protein for the vaccine is scalable, and that mass-producing the microneedle array involves spinning the protein-sugar mixture into a mold using a centrifuge. Advantages of the vaccine, dubbed PittCoVacc, are that (like other MAPs) it does not require a cold chain for storage and that it maintains its potency after being sterilized with gamma radiation. The researchers are in the processing of submitting an investigational new drug application to the United States Food and Drug Administration (FDA).

PATH, a nonprofit, global health organization has been investigating transdermal drug delivery patches for more than 10 years and is in the middle of a four-year initiative, through its Microarray Patch (MAP) Center of Excellence, to accelerate development for global health needs, such as vaccines and essential medicines, in low- and middle-income countries. The group says that developing scalable, automated, good manufacturing practice (GMP) processes is crucial for success (4).

"Although microneedle arrays are being used commercially in some cosmetic applications in Asia, the technology is not yet commercial for vaccines. Optimization of equipment and manufacturing processes is crucial for producing these systems in the large quantities and reasonable costs needed for clinical studies and vaccination campaigns," adds Stefan Bernsau, sales director for Needle Technology at Harro Höfliger (HH), which develops and manufactures various types of production and packaging equipment for pharmaceutical companies, the medical device industry, and other industries. The company is working with various organizations and partners to develop microneedle array patch (MAP) technology, and in January 2020, Harro Höfliger and PATH hosted a workshop on MAP manufacturing attended by MAP developers and representatives from the World Health Organization, UNICEF, the Bill & Melinda Gates Foundation, and the Gavi Vaccine Alliance. *Pharmaceutical Technology Europe* spoke with Bernsau about some of the considerations for MAP manufacturing.

Advantages of MAPs

PTE: What are some of the advantages of using MAP for vaccines?

Bernsau (HH): Vaccine delivery faces several challenges that can be addressed with MAP technology. A significant issue is that low- and middle-income countries often do not have a clear cold chain, which is necessary for transport and storage of liquid, injectable vaccines. MAPs do not require a cold chain. Another concern is that a significant number of people have a fear of

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regular injection needles and the associated pain. Microneedles in a patch form eliminate the pain. In addition, MAPs can be administered without skilled healthcare workers, by trained workers or potentially by self-administration. This advantage is beneficial for developing countries that lack skilled healthcare providers. Recently, it is also being thought of as a benefit for all countries for use in pandemics, where patients could potentially have the vaccine delivered for self-administration, thus avoiding the need for people to go to doctors' offices or hospitals.

Microneedle array patches are being investigated for a vaccine to fight COVID-19.

Manufacturing considerations

PTE: What are the biggest challenges in microneedle patch manufacturing and what are some best practices for addressing these challenges?

Bernsau (HH): Companies developing products at the laboratory scale are looking at issues such as dosing (either by coating or filling) and drying. At the commercial scale, however, automation is crucial for obtaining output at an appropriate cost, with a reasonable total cost of ownership. As a machine manufacturer, we want to join in the development process as early as possible so that we can provide input to developers for how to optimize the process for commercial automation.

One of the constraints for vaccine manufacturing is that most cannot be terminally sterilized, therefore they must be produced in a sterile environment. One of the keys for sterile production is material flow through the processing line: raw materials must be brought into the machine; various automated steps are performed; then the product must be taken out of machine. Sterile environments can include isolators or various types of barrier technology, depending on the cleanroom setup.

Dosing of the API, either into the hollow mold or as a coating, must be done in a combination of high precision and with high output. These are very small doses, and different dosing technologies are used to obtain the specific tolerances needed for a particular process. There are some new developments in the market for dosing. VAXXAS, for example, has developed their own high-speed and high-precision dosing technology that is similar to ink-jet printing technology. As another example, scraper technology can be a solution for dissolvable microneedles, depending on the viscosity and the physical characteristics of the liquid or the vaccine.

For the automation of the lines, we use dedicated servo-driven units because the parts being handled are so small—the whole array may be about the size of a penny, for example. Robotic arms can cause turbulence in the airflow, which can be a problem regarding the aseptic requirements of such small, precise units. Another proven technology is a so-called 'walking beam,' which uses pick-and-place handling for small and delicate parts inside an isolator.

PTE: What are some best practices for inspection of MAPs after manufacturing?

Bernsau (HH): Several quality attributes need to be inspected, particularly that the required amount of drug is there (either coated or filled). Inspection of microneedles is more difficult than syringe inspection because of the small size of the microneedles. Cameras are used to view the top and side. Other critical pieces are the light source, appropriate software, and hardware for fast computing speed. A best practice is 100% inspection of each part, but a challenge is the speed and an eventual reflectance of image acquisition to accomplish this.

Packaging requirements

PTE: What are the requirements for packaging of microneedle patches?

Bernsau (HH): To a large degree the microneedles are fragile; they are typically packaged in an applicator, with a rigid container to protect the needles. The patch must also be packaged in a sterile environment. After primary packaging it must also be packaged for transportation. An advantage of MAPs is that temperature is not as much of an issue, compared to traditional liquid vaccines, because the MAPs vaccine is dry.

"After primary packaging [MAPs] must be packaged for transportation. An advantage of MAPs is that temperature is not as much of an issue, compared to traditional liquid vaccines, because the MAPs vaccine is dry."

—Stefan Bernsau, Harro Höfliger

Outlook

PTE: Do you foresee in-country, for-country production with MAP technology?

Bernsau (HH): It is far too early to know whether production in developing countries is feasible, because the initial investment in these lines is high and a lot of technical knowledge and skill are needed to run these lines. However, some organizations are looking at this possibility, and the current pandemic may cause more governments globally to think about in-country production.

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Good Manufacturing Practices: Aseptic and Sterile Processing

Organizations involved in aseptic and sterile processing activities must make an effort to comply with current good manufacturing practices.

Lauren Lavelle

Taking the proper steps to comply with current good manufacturing practices (cGMPs) for aseptic and sterile processing in an efficient and effective manner is necessary for pharmaceutical manufacturing facilities and labs. *Pharmaceutical Technology Europe* spoke with Ulrich Herber, senior global product specialist manager for Microbial Solutions, Charles River, and Sade Mokuolu, group product compliance manager, Watson-Marlow Fluid Technology Group, about best practices for GMPs in aseptic and sterile manufacturing and in quality control laboratories.

Compliance

PTE: Why is it important for organizations engaged in aseptic/sterile processing activities to practice GMPs?

Herber (Charles River): First and foremost, practicing GMPs ensures a safe, efficacious, and high-quality product that preserves the safety of the end-user: the patient. However, practicing GMPs also ensures that the risk of contaminating the product is reduced or detected and controlled quickly. This ultimately maximizes operational efficiency, eliminates waste, and improves an organization's bottom line.

Mokuolu (Watson-Marlow): cGMPs are important to ensure that pharmaceutical products meet high quality standards and are safe and effective. As producers of high-value medicinal products, non-compliance with regulations could risk consumers' health, failing to achieve regulatory approvals, and significant loss of revenue. It is therefore important to not just comply with cGMP regulations, but also be able to demonstrate that you have. GMPs are necessary to demonstrate that there are good control procedures built in during the manufacturing process to prevent errors that cannot be eliminated through quality control of the finished product. Without GMPs, it is impossible to be sure that every unit of medicine is of the same quality as the units of medicine tested in the laboratory.

PTE: How challenging or difficult is it to comply with GMP regulations?

Mokuolu (Watson-Marlow): GMP regulations are well defined and focus essentially on the five P's: People, processes, procedures, premises, and products. Once there are appropriate controls and ongoing verification of these aspects has been established, it should not be difficult nor challenging to comply to the GMP regulations. The heart of GMP is the establishment of well-written procedures for each operation. These written procedures give us the controls necessary to minimize the chance of mix-ups and errors in manufacturing a product. By carefully following written procedures, it serves to provide evidence of compliance with the GMP regulation, but more importantly, it helps to ensure the consistent quality of drug products. Even though the GMP regulations are well defined, following them precisely can be a challenge for a number of companies, as frequently highlighted in the warning letters (known as 483s) that FDA [the US Food and Drug Administration] publishes regularly.

Herber (Charles River): By and large, GMP regulations are not new to the pharmaceutical industry. Many companies not up to date with current best practices can mistakenly find it overly challenging to comply, but this is not necessarily the case; in fact, it is often easier to exceed minimum requirements very simply.

For example, many new GMP requirements focus on improving the integrity of quality testing data while also reducing subjectivity in analysis and reporting. Many labs are quick to accept the first, most simple option as a solution: applying the 'four eyes principal.' Unfortunately, while straightforward to implement, it is not scalable nor is it a true solution. What if the lab performs more tests of the assays requiring two analysts to score the result, and thus, require more time from not one, but two analysts? What if the analysts get different results? What if both analysts get the answer wrong? In many cases, the cause for non-GMP compliance is the test method itself being outdated. These can often be solved by modernizing

the traditional test method with rapid or alternative methods and using instrument-based analyses.

Additionally, microbial identification techniques continue to evolve as more information becomes known due to advances in science. Many traditional, bench-based methods for identification, such as gram-stain or phenotypic identification, have been proven to be incapable or error-prone in identifying certain species or groups of closely related species, such as *Burkholderia cepacia* complex. Many labs feel that they must continue to use outdated techniques because implementing a new technology can be cost-prohibitive and resource intensive, even though outsourcing their identification to a specialized identification lab is a proven, reliable, and more cost-efficient option.

"GMP regulations are not new to the pharmaceutical industry."

—Ulrich Herber, Charles River

Instruments

PTE: What equipment/instruments does your facility rely on when coordinating aseptic/sterile processing activities?

Herber (Charles River): Utilizing rapid and/or alternative test methods can be a solution that labs can rely on for aseptic/sterile processing. These instruments allow a lab to automate many processes including the assay, the results analysis, and data reporting. Many alternative and rapid methods can replace traditional methods that can pose a risk of non-compliance with GMPs. These include the use of FDA-licensed, cartridge-based LAL [Limulus amebocyte lysate] test methods and instruments to replace antiquated gel-clot methods, or using ATP [adenosine triphosphate] bioluminescence to replace visual turbidity analysis in final product sterility testing. Both of these technologies replace traditional, error-prone assays with automated technology.

Mokuolu (Watson-Marlow): A recent amendment to European guidelines for the manufacture of sterile products, Annex 1 includes the requirement to reduce contamination sources in aseptic processing (1). It details that wherever possible, interventions should be performed aseptically and how the risk of product contamination could be reduced by the use of pre-sterilized closed systems.

Best practices

PTE: How do you suggest other organizations improve/enhance their aseptic/sterile processing activities (specifically, workflow, day-to-day operations, etc.)?

Herber (Charles River): Day-to-day improvements to lab workflows are easily achievable through implementing more automation in the microbiological quality control lab. This speeds the time to result of many assays, which creates higher throughput in the lab's general operation. It also reduces stress and anxiety on your lab's staff by reducing the chances of error, the need for retests, and the potential burden of performing investigations for root cause. These alone can greatly improve the overall value, utility, and employee satisfaction in an organization.

Mokuolu (Watson-Marlow): The draft version of Annex 1 has placed a significant emphasis on applying appropriate contamination controls during drug product manufacturing. It describes how contamination risks could be minimized through the use of pre-assembled sterilized equipment.

A further way of enhancing aseptic/sterile processing is to reduce risk through automation. A particular critical unit operation during biomanufacturing is the final filling of the drug product. To this end, equipment such as an automated vial filler and capping unit could be used to provide an aseptic environment and control of process steps.

PTE: Are there any best practices you can recommend for new organizations (e.g., at a start-up), specifically on how to practice and comply with GMPs?

Mokuolu (Watson-Marlow): For new companies, there are a number of ways to comply with GMP regulations. The increasing use of pre-sterilized systems such as single-use assemblies offers a number of advantages that include: no cleaning validation; easy product changeover, particularly for multi-product facilities; and no cross contamination. Additionally, working with a reliable and trusted supply partner with knowledge of GMP regulations means that prevalidated components are easily incorporated into processes and they can also provide effective support and verification of their supply chain.

"There are a number of ways to comply with GMP regulations."

— Sade Mokuolu, Watson-Marlow

Herber (Charles River): Startup organizations often mistakenly feel they don't have the expertise or capacity to implement rapid methods in the beginning and trust the ease and comfort of traditional methods. However, they fail to realize that as a startup, they have the perfect opportunity to innovate and use modern methods in the beginning, rather than try to overcome inertia and reliance on these methods later. Investing the time to gain the knowledge and experience of using the best available methods early will set up startups for success for years down the road. Using rapid, alternative methods not only ensures GMP compliance right out of the gate, but ensures successful business operations by optimizing production, improving product quality, and reducing risks.

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Complying Confidently? Learning Lessons from Nitrosamine Impurities

Industry must never become complacent about the safety of drug products and should seek to continually perform surveillance even if the drug is well-established.

Felicity Thomas

In 2018, regulatory authorities were alerted of nitrosamine impurities in valsartan that had originated from an API manufacturer based in China (1). "Within Europe, the issue is generally considered to be centred around a report of a non-good manufacturing practice (GMP) compliance (2), which was submitted by the competent authority of Italy in September 2018," explains Diego Martinez, senior manager CMC at PharmaLex.

This report pertained to an inspection of Zhejiang Huahai Pharmaceutical (a Chinese manufacturer of a valsartan API), which led to dozens of recalls of various sartans medicines, Martinez continues. Furthermore, the US Food and Drug Administration (FDA), after completing its own inspection, issued an import alert on Zhejiang Huahai Pharmaceutical (3) and then, a few months later, a warning letter (4), iterating the deviations from current GMP for APIs. Other ingredients manufacturers have also been issued warning letters by FDA, including Aurobindo Pharma, Hetero Labs, Lantech Pharmaceuticals, Torrent Pharmaceuticals, and Mylan (5).

"The change that resulted in the formation of the potential carcinogen, N-nitrosodimethylamine (NDMA), was introduced into the manufacturing process of the valsartan API in approximately 2012 or earlier, but was not uncovered by regulators until more than five years later," says Martinez. "Since its discovery, recalls have been conducted by a variety of firms that purchased the contaminated API from either the same or a limited number of API manufacturers."

In September 2019, the same potential carcinogen (NDMA) was identified by FDA in Zantac and other generic versions of the drug (ranitidine) (6). "The contaminants have also been found in low levels in batches of pioglitazone produced by Hetero Labs (7), indicating the potential for the carcinogens to affect products other than sartans and ranitidine medicines," adds Martinez.

Actively investigating contaminants

The European Medicines Agency (EMA) has been actively investigating the origin and prevalence of nitrosamine contaminants in human medicine, reveals Martinez. "Both EMA and FDA have established limits for nitrosamines in the affected medicines," he says.

In a recent publication from EMA on the topic, requirements on manufacturing authorization holders (MAHs) have been imposed (1). These requirements include an evaluation of all finished pharmaceutical products (FPPs) that contain chemically-synthesized APIs by MAHs to assess any potential presence of nitrosamines. "EMA has requested that MAHs complete the review before 1 October 2020," asserts Martinez.

"Revisions are being made to the *European Pharmacopoeia* to the drug substances monographs for the sartan series to include testing for nitrosamines," Martinez continues. "In addition, the general monograph for APIs (General monograph 2034) is under revision and will also include appropriate tests."

Similarly, FDA is actively working to identify and recall medicines that have nitrosamine levels above interim acceptable limits and regularly updates its published list of angiotensin II receptor blocker (ARB) products with respect to nitrosamine content (5). "However, both EMA and FDA have emphasized that the risks associated with an abrupt discontinuation of ARB products, such as stroke, far outweigh the low risk associated with continuing to take medications with nitrosamine impurities," Martinez adds.

Complete consideration required

"As a result of investigations into the presence of nitrosamines, it is apparent that a complete consideration of potential nitrosamine contamination in an FPP must be broader than whether sources of amines and nitrites are concurrently used in the preparation of the API," says Martinez. "Manufacturers of all FPPs should

be assessing their products for any circumstances that might inadvertently lead to nitrosamine content and taking steps to mitigate these risks."

Manufacturers must also make all relevant information, required for an appropriate risk assessment, available to MAHs, Martinez explains. "For those products that have active substance master files (ASMFs) and certification of suitability to the monographs of the *European Pharmacopoeia* (CEPs), MAHs remain responsible for ensuring that robust risk evaluations have been appropriately performed prior to taking responsibility for the quality of the API and medicinal product," he adds.

These robust evaluations, either performed by the MAH or API manufacturer (including ASMF or CEP holders), must be performed in accordance with Article 46 of Directive 2001/83/EC, Martinez stresses. "MAHs should apply for a variation in a timely manner to introduce any required changes," he notes. "The variation should contain information on amendments to the marketing authorization—i.e., module 3 (3.2.S and 3.2.P), the active substance master files (ASMF), or certificates of suitability (CEP)—that are necessary to amend the method of manufacture or control of the active substance and/or finished product."

General recommendations

Health authorities have issued general recommendations that MAHs of all FPPs evaluate whether or not nitrosamines are present in products that contain chemically synthesized APIs. "Although nitrosamines are not expected to form during the manufacture of the vast majority of medicines, the possibility of cross contamination or unintentional introduction of amines and nitrites has prompted the request for companies to undertake this precautionary review," Martinez confirms. "MAHs together with API and finished product manufacturers are required to perform risk evaluations using quality risk management principles, as outlined in [the] ICH [International Council for Harmonization] Q9 guideline. The principles described in [the] ICH M7 guideline in relation to toxicology assessment, control strategy, and changes to the manufacturing processes for active substances should be applied."

In any products that are likely to include nitrosamines, it is recommended that MAHs perform confirmatory testing on the API, the FPP, or both, Martinez iterates. However, as the low levels at which nitrosamine impurities occur can prove problematic in testing, several test methods have been published for assistance in this area.

"FDA has recommended the use of a liquid chromatography–high-resolution mass spectrometry (LC–HRMS) method when testing ARB drug products as a result of the lower temperature conditions of the method (8)—higher temperature conditions of some test methods may cause the sample to generate NDMA," says Martinez. "Similarly, the Official Medicines Control Laboratories (OMCLs) Network of the Council of Europe has also published several methods (9), which may be used when testing for nitrosamines in various drug substances."

Lessons to be learned

"In my opinion, the nitrosamine impurities issue could be useful as a 'lesson learned' that following procedures is important," Martinez says. "It is beneficial to view the issue as a way of enhancing the value of risk assessment tools, particularly from a regulatory standpoint. These tools have been frequently used in quality processes as they employ scientific data to direct the best course of action and now they should be implemented in regulatory procedures more regularly too."

European authorities are conducting an exercise to establish what lessons can be learned from the identification of nitrosamines in sartans. At the time of writing, this 'lesson learnt' group is in the process of finalizing its recommendations on how the presence of impurities can be prevented and better managed in the future (10).

"Several conclusions can be drawn from the experiences with sartans," summarizes Martinez. "We need continuous and continuing safety surveillance of drugs—even very old drugs and those presumed to be quite safe. The safety profile of a drug is never fully known. We cannot become complacent. A high level of suspicion must be maintained particularly with odd and unexpected safety signals and serious adverse events. Late appearing safety issues can be totally unexpected."

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Starting a Career in the Bio/Pharmaceutical Industry

Having a better understanding about compliance will be of benefit when looking for a job or for furthering one's career, says Siegfried Schmitt, PhD, vice-president, technical, Parexel Consulting.

Q. While working on a variety of projects in three different continents, I had the opportunity to meet and work with young, enthusiastic newcomers to the industry. They were from a variety of different professional backgrounds, including pharmacists, engineers, and chemists. During our conversations, most of them asked the following types of questions:

- The college or university I graduated from did not provide courses on compliance or industrial operations—how can I fill this gap?
- Though I applied for many positions, I have been unable to find a permanent position yet—how can I improve my chances?
- On the Internet, I found several courses on good manufacturing practices (GMPs), for compliance experts, or similar. These are relatively expensive. Are they worth investing for someone like me (a beginner)?
- Should I work my way up within a particular department or would it be better if I gain experience in different departments?

A. The following are not exhaustive or the only answers to these questions, but they will give some insight.

It is true that few graduates have seen industrial operations by the time they graduate, but that doesn't mean that they don't come equipped with many of the basic skills needed in the industry, such as team working, presentation skills, analytical thinking, and the ability to self-study. Companies will provide training, as a minimum on the applicable and relevant internal processes and procedures, which will cover both the operations and the compliance side of the business. A lot will be learning on the job, from peers and often also from mentors.

Finding your first permanent job can be a frustrating experience, but persistence usually pays off. Gaining experience through temporary voluntary engagements, placements, or positions is what helps improve the chances for long-term or permanent employment. And don't forget to network through portals such as LinkedIn, Bing, or similar sites that have a good reputation with industry and job agencies. Also, it's important to write a succinct and well-thought-out curriculum vitae, and there is a lot of great advice available for free online on how to do this. The Internet is the place to research jobs, but often also for potential employers to find the right candidate.

Having a better understanding about compliance will surely be of benefit, whether looking for a job or for furthering one's career prospects. Whether you are lucky enough to have your employer pay for external courses, be subsidized (e.g., by a state job centre), or have to pay yourself, in all cases you should scrutinize the courses offered:

- How relevant are they to your current or prospective work?
- Do they provide references? Is there feedback available?
- Are the certificates merely proof of attendance or are they widely recognized by the industry?
- Is it just classroom learning or is there also a practical element?
- Do they provide comprehensive documentation?

The answers to these questions will help you determine if the course is right for you. For example, if you want to become a certified auditor (be it for GMP or ISO 9001), you will have to pay for a course with an approved training company. If however, you want to get a basic understanding of the freeze-drying process, you will easily find free tutorials online. Should you need hands-on experience, then training courses offered by universities or industry associations with in-house training centres will be the right choice.

Switching careers

What if you have been in the industry for a while, but would like to change positions and/or area of expertise? Very often this is less of a question of opportunity, but more of a question of an individual's preferences. There are equally excellent subject matter experts who never strayed from their vocation (say regulatory affairs, quality control, or manufacturing) and who are perfectly happy in their jobs, and there are those who worked in different departments to become more universal experts. Pharmaceutical companies probably look more for experts in a particular subject, whereas service providers, such as consultancies or contract research organizations, may have a need for experts with more varied backgrounds.

We may not always find the job we want, but we can always learn from what we do, and it will always be a beneficial personal and job experience. **PTE**

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