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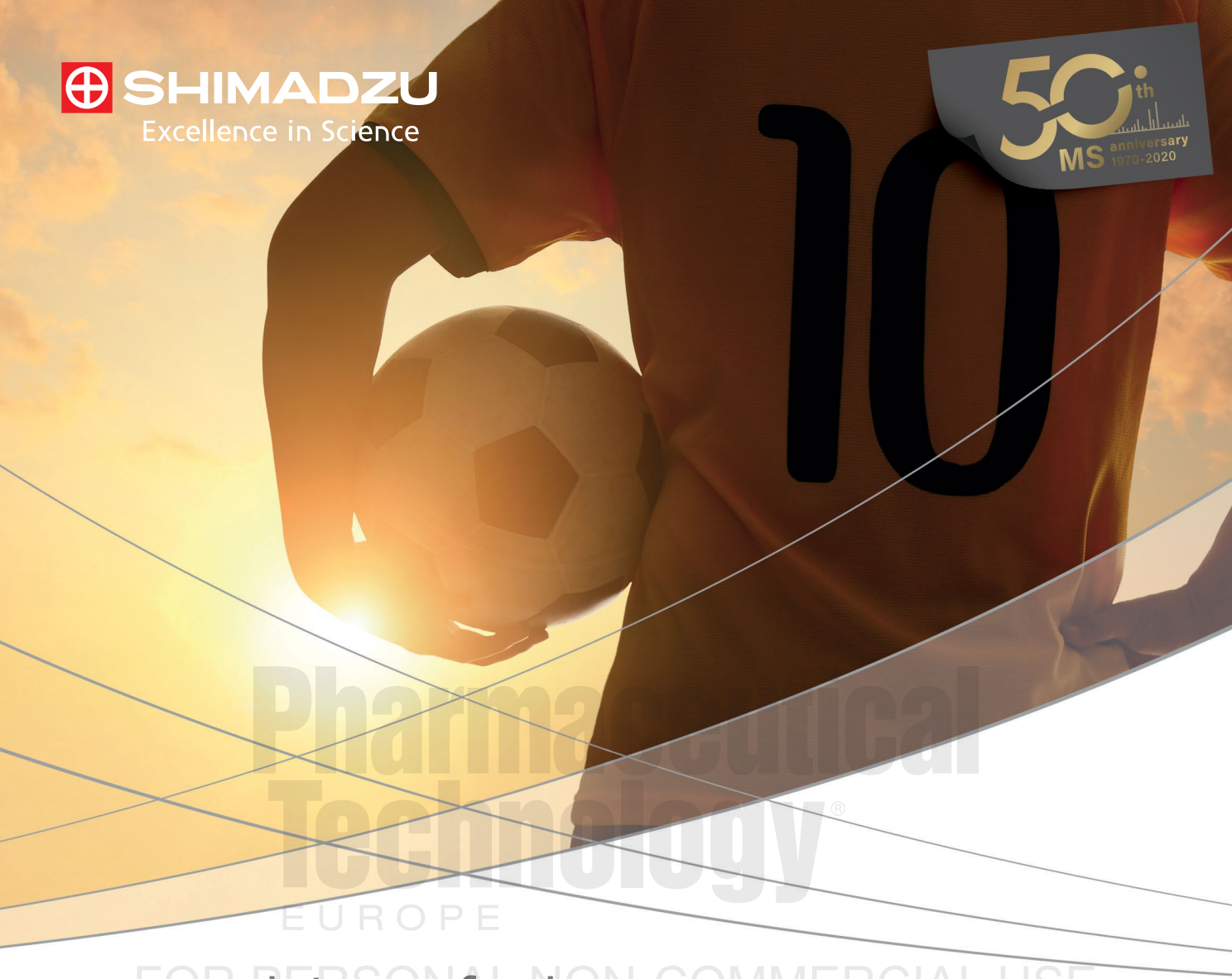

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Of Serious Concern?

Amid the rapidly rising cases of a novel coronavirus, concerns are being raised over preparedness and potential disruptions to the pharma supply chain.

As the rate of infection of a novel coronavirus, first identified in Wuhan, China, rises rapidly and more countries report confirmed cases, pharma companies are rallying to find effective treatments to combat the virus. However, concerns regarding the global supply chain are also being raised, as a vast proportion of APIs are sourced from suppliers located in China, a country currently going into lockdown.

A global health concern

The new coronavirus, 2019-nCoV, is believed to have originated from animals, and symptoms include respiratory issues and fever. As a result of the 2019-nCoV being viral in nature, antibiotics are of no use, and human-to-human transmission is occurring, which can aid the spread of the virus on a global scale.

"Stopping the spread of this virus both in China and globally is [the World Health Organization's] WHO's highest priority," said Dr Tedros Adhanom Ghebreyesus, director-general of WHO in a press release (1). "WHO is working closely with the [Chinese] government on measures to understand the virus and limit transmission."

Vaccine development programmes underway

The Coalition for Epidemic Preparedness Innovations (CEPI) revealed in a press release that it has initiated funding of three programmes aimed at the development of vaccines against 2019-nCoV (2). Building on existing partnerships with Inovio and the University of Queensland (Brisbane, Australia), in addition to a new partnership with Moderna and the United States National Institute of Allergy and Infectious Diseases, CEPI hopes to leverage rapid response platforms that are already being worked on to advance candidates into the clinical setting as quickly as possible. Additionally, several US drug makers (including AbbVie and Johnson & Johnson) have been sending drugs that are approved to be used in the treatment of HIV to be used off-label in efforts to treat those infected, as reported by *The Wall Street Journal* (3).

However, in an interview with CNBC, Vas Narasimhan, CEO of Novartis, projected that it will take more than a year to find a new vaccine for 2019-nCoV (4). "The reality is that it will take over a year, in my expectation, to really find a new vaccine for [2019-nCoV], so we really need to use epidemiological controls to really get this situation in a better place," he said in the interview.

Supply chain at risk?

Despite the lengthy time projected by Narasimhan for a new vaccine to be found for 2019-nCoV, he also stressed that, for Novartis at least, he does not expect any disruptions in the supply chain and for the company to continue to be able to deliver medicines in the coming months (4). However, according to the Medicines and Healthcare products Regulatory Agency (MHRA), China accounts for 40% of the global production of APIs (5), and so, as more restrictions are made on transportation of materials in and out of the country, supply disruptions may become apparent later down the line.

The recent 2019-nCoV infection is not the only issue that has sparked concern around the monopoly China seems to have on the pharmaceutical ingredients market. Apprehension on the stability of the pharma supply chain has been further exacerbated by the nitrosamine impurities issues found in sartans, that are still being investigated by regulatory bodies, and substandard vaccine doses that had been sold in China in 2018 (6).

If the SARS outbreak, which occurred in 2002 to 2003, is anything to go by, then it is probable that global markets will be affected. Ultimately, as was the case nearly 20 years ago, it will be the speed at which the rate of this novel coronavirus infection can be contained that will have an impact on the recovery of the markets and supply chains.

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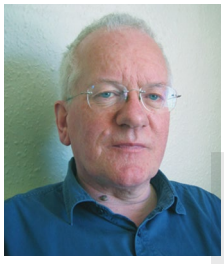
Brexit: What Happens Next for Pharma?

The UK and Europe are entering a transitional period, which will involve negotiations across the board, including those on the pharma regulatory landscape.



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The United Kingdom and the European Union were scheduled to start negotiations in February 2020 on a permanent trading relationship in pharmaceuticals and other products after the UK finally departed the EU on 31 January 2020. Since the UK's referendum vote in June 2016, Brexit has eventually led the way to a year's transition period for the talks on an EU–UK Free Trade Agreement (FTA).

Brexit also ended—at least for a while—three and half years of painful uncertainty during which there was a big threat of a 'no-deal' scenario on immediate issues, such as financial compensation and the position of around three million EU citizens who reside in Britain. No-deal would have triggered the immediate imposition of customs barriers leading to shortages of medicines both in the UK and the EU.

Industry priorities

For the pharma industry, the FTA aims to be a bold attempt to supply the basis for a collaborative regulatory framework to prevent large regulatory gaps opening up between the UK and European medicines sectors. But few experts believe that a year is long enough to thrash out such a complex arrangement, particularly in a such an intricately regulated sector such as pharmaceuticals. In the summer of 2020, the UK government has the choice of extending the transition by at least another year (1), but it has vowed not to take this opportunity.

For the industry, the priority will be that by 1 January 2021, when the transition will have ended and Brexit at last fully completed, there will be sufficient agreement on basic trade rules to ensure a smooth flow of medicines between the UK and the 27 EU member states. The worse-case scenario would be a breakdown in negotiations or a transition that ends without a deal of any sort. The EU–UK trade in pharmaceuticals and other products would then be governed by the rules of the World Trade Organization (WTO), which would seriously disrupt medicines supplies.

The UK is a major centre of pharmaceuticals production in Europe, representing both a leading exporter and importer of medicines within the region (2). The EU accounts for around half of the country's pharmaceutical exports and close to three quarters of pharmaceutical imports (3).

Currently, 45 million patient medicine packs are supplied each month from the UK to EU member states and three European Economic Area (EEA) countries of Norway, Iceland, and Liechtenstein (4). The EU/EEA in turn exports 37 million packs per month to the UK, according to figures from the European Federation of Pharmaceutical Industries and Associations (EFPIA) in Brussels (4).

As long as EU regulations continue to apply to the UK during the transition, the stream of medicines, active ingredients, intermediates, and other raw materials between the EU and the UK will continue. Quality testing certificates will be recognized by the EU and vice versa, as will manufacturing and distribution licences. Marketing authorization holders (MAHs) will be able to remain based in the UK to have access to EU markets. In the context of international agreements, including mutual recognition agreements (MRAs) such as those covering good manufacturing practice (GMP) inspections, the UK will be treated as an EU member state.

The big difference will be that the country will be a taker of EU regulations without any involvement in decisions in the drawing up or approval of new regulations or their amendments. UK regulators are no longer allowed to be rapporteurs or reference states for the co-ordination of approvals and mutual recognitions under the decentralized licensing procedure. In most cases, they will not be able to act as observers at meetings assessing marketing authorization applications.

"Decisions taken on marketing authorizations will be applicable in the UK," a spokesperson for the UK Medicines and Healthcare products Regulatory Agency (MHRA) told *Pharmaceutical Technology Europe*. "The UK is not permitted to lead work, such as marketing authorization assessments, and is only allowed to attend decision-making committees 'exceptionally'," they added.

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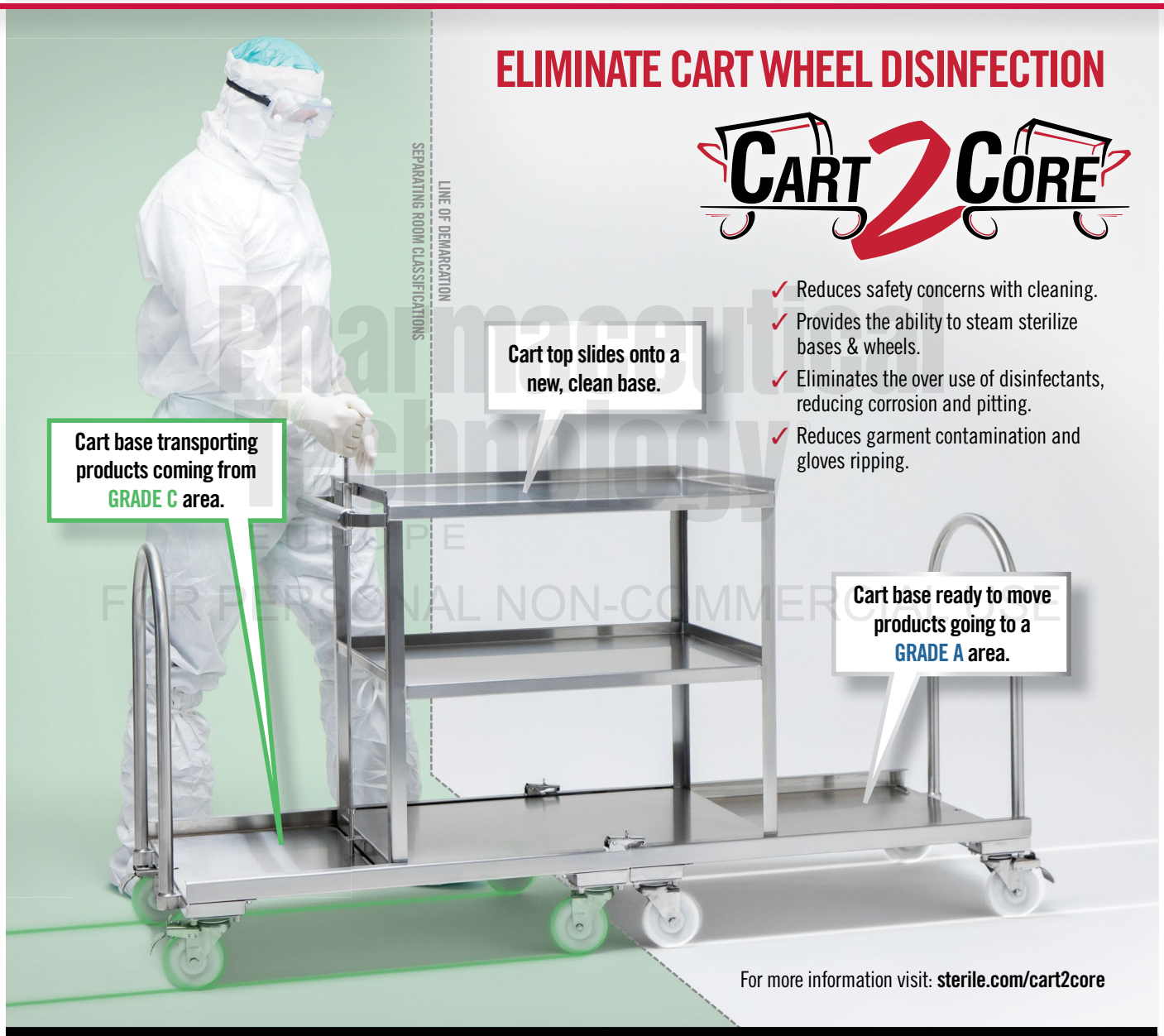

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Transitional preparations

UK-based pharma companies have been urged by the UK government or their trade association to use the transition to reorganize their distribution and supply operations in preparation for the full implementation of Brexit at the beginning of next year. EU pharma companies are being advised to take similar steps with regard to their exports into the UK. For many companies, particularly multinationals, decisions on these changes will have been taken last year because of the threat of a no-deal Brexit.

One key aspect after the transition end is the location of batch testing and the base of a company's qualified person responsible for quality control, both of which will have to be in an EU member state. Also, the locations of MAHs have to be moved to the EU (5). Preparations will have to be made for the approval, after the implementation period, of variations to centralized and decentralized authorizations if there have been changes to manufacturing and supply operations and to the labelling of products.

The medicines industry in Europe, led by EFPIA, wants, with the support of the UK pharma sector, to keep pharmaceuticals regulations in the region as closely aligned as possible (4). This may not be achievable because, for the UK government's ruling Tory party, a fundamental objective of Brexit is the introduction of regulations which diverge from those of the EU.

The government's aim with the UK pharma industry is to strengthen its leadership in basic research and product development with the help, if necessary, of the country's newly acquired legislative freedom. This could be used to boost its biopharma clusters in areas with close links to universities like Oxford, Cambridge, and London, as well as hospital-based centres of excellence with their own manufacturing capacity.

In EFPIA's latest study of the performance of the European medicines sector, the UK pharma industry accounted for around 15% of the European industry's R&D expenditure. It was the second biggest spender behind Germany in the EU (2). The UK is among the top five locations in Europe for clinical trials. Its two main pharmaceutical companies—GlaxoSmithKline and AstraZeneca—have been among the leaders in all European sectors for R&D collaborations between industry and academia.

To encourage breakthroughs in technologies like gene therapy, the UK government could give hospitals more regulatory opportunities to develop decentralized systems for the manufacture of bedside treatments. This would contrast with the European Commission's seeking of ways to tighten up controls on hospital-based drugs production (6).

With the development of new production processes, the MHRA may make use of licensing agreements which after approval would be conditional on improvements in the quality, safety, and efficacy of new manufacturing technologies.

The UK government, which in December 2019 won a general election by a substantial majority, has promised to replace EU research funds for UK projects with its own money (7).

UK is not alone

In the European pharma sector, the UK is not alone in being a large player able to pursue, if necessary, its own regulatory strategies. Switzerland, which is among the biggest R&D spenders on medicines as well as being a major pharmaceuticals producer and exporter in the region, is not a EU member.

Between them, the UK and Swiss medicines industries account for around a third of the European pharma industry's R&D expenditure and around a quarter of total output (2). Some analysts have predicted that the UK will follow the Swiss model of having close ties with the EU in some areas while in others taking advantage of its regulatory flexibility.

There has already been one big Anglo-Swiss collaborative initiative in product development. Novartis, which, with Roche, is the big Swiss multinational player in the global market, announced in January 2020 a planned collaboration with NHS England, the English arm of the UK's state-run National Health Service, for the development of the company's anti-cholesterol drug inclisiran (8).

The UK's strategy with Brexit is to be less reliant on Europe's pharma market by becoming a bigger global player. In parallel with the negotiations on the EU–UK FTA, the country is negotiating an FTA with the US to be followed by planned talks on trade deals with other non-EU countries such as China. But it faces a dilemma in reconciling its desire for regulatory divergence with the EU with the growing trends for regulatory convergence in the world's medicines sector.

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Biopharma Analysis Benefits from New Technology and Methods

Analytical solutions are improving for raw material testing, process development, drug product release, and more.

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

Effective analytical methods are essential for the successful development and commercialization of both small- and large-molecule drug substances and drug products. As the complexity of both biologic and chemical drug substances increases, analytical methods must evolve as well. New analytical techniques and methods are therefore crucial to the fast-moving bio/pharma industry.

"Faster, more efficient techniques will give companies an advantage as their products move through the pipeline," asserts Robin Spivey, director of analytical research and development, Cambrex High Point. Techniques that are more sensitive and more accurate will, she says, better position a company for regulatory acceptance as long as they are willing to help pioneer the techniques. In addition, such companies will be seen as being at the forefront of the industry.

Major strides in analytical methods

Some of the most noteworthy advances in analytical methods involve the application of mass spectrometry (MS) for process development and product release of both biologics and synthetic drugs, the enhancement of chromatographic techniques, particularly liquid chromatography (LC), microcrystal electron diffraction, and techniques designed for use as process analytical technology (PAT).

For biopharmaceuticals, MS was initially limited to use for protein characterization to provide supplemental information for regulatory filings, according to Amit Katiyar, director of analytical and formulation development for bioprocess sciences at Thermo Fisher Scientific. Process release/stability testing continues to largely depend on conventional analytical methods such as LC, capillary gel electrophoresis (CGE), imaged capillary isoelectric focusing (iCIEF), and enzyme-linked immunosorbent assays (ELISA) due to their simplicity and wide adoption in quality control (QC) labs.

Inclusion of biosimilars, complex non-monoclonal antibody proteins (e.g., fusion proteins), bispecifics, and combination products in the product pipeline, however, is presenting challenges due to the inability to gain a thorough understanding of these molecules using platform methods. "Most of the time, platform methods may not be able to provide the information required to develop and commercialize complex biomolecules. In these cases, MS-based methods are being used for process development and as identity and release/stability indicating methods," Katiyar observes.

In addition to using peptide-mapping principles in multi-attribute methods (MAMs), major biopharmaceutical companies are now using MS-based identity methods to release biologic drug

substances and drug products. "This approach will provide the opportunity to gather more information on the performance of MS instruments in QC labs that can then be used for implementing MS technology for process development, release, and stability testing," says Katiyar. The current approach for regulatory filing, he adds, is to use a combined package of conventional methods and MS methods to gain more confidence from health authorities and be able to present a future case for submissions based only on MS data.

For Da Ren, process development scientific director at Amgen, MAM is probably the most important emerging analytical technology that has been used in process development and release and stability testing of therapeutic proteins. "MAM is an LC/MS-based peptide mapping assay. Unlike profile-based conventional analytical assays, which focus on whole or partial proteins, MAM can identify and quantify protein changes at the amino acid level and can provide more accurate information on product quality related attributes," he explains. Notably, MAM is capable of replacing four conventional assays including hydrophilic interaction liquid chromatography for glycan profiling, cation exchange chromatography for charge variant analysis, reduced capillary electrophoresis-sodium dodecyl sulfate for clipped variant analysis, and ELISA for protein identification, according to Ren.

In the case of small-molecule drug development and commercialization, MS detection systems are no longer considered just research tools and are becoming more widely used for routine QC testing, for example determining extremely low level impurities such as genotoxic impurities/potential genotoxic impurities, according to Geoff Carr, director of analytical development in Canada with Thermo Fisher Scientific.

"These advances are very likely in response to new regulatory guidelines issued by agencies such as the US Food and Drug Administration (FDA) and the European Medicines Agency, but also as a result of specific problems that have occurred in the industry, such as recent concerns regarding observations of N-nitrosamine residues in sartans," Carr explains.

Efficiency gains for analytical workflows

Changes in analytical workflows have the potential to impact productivity and efficiency but may also create challenges depending on the nature of the modifications. These changes may also originate as the result of new technology or new processes and approaches.

As an example of the former, Heewon Lee, director of analytical research and quality systems in chemical development US for Boehringer Ingelheim Pharmaceuticals, points to material identification using Raman spectroscopy as a technique that has impacted analytical workflows associated with small-molecule API manufacturing. "This technique is now mature, and several companies have launched products that are user-friendly and GMP [good manufacturing practice]-compliant. Benefits are gained because this method can be used to identify raw materials, intermediates, and APIs in the process area. QC personnel can then release batches based on the Raman data acquired, streamlining the analytical workflow," she explains.

For biologics, using MAM through process development and release and stability testing is a revolutionary analytical workflow, according to Ren. "The continuous monitoring and control of product quality attributes at the amino acid level during product and process characterization as well as release and stability testing enhances the understanding of biotherapeutic products and processes," he asserts.

One driver leading to changes in analytical workflows is the desire to achieve greater efficiencies and thereby reduce operating costs, according to Carr. One approach that many pharma companies have taken, he notes, is to implement operational excellence initiatives within laboratory operations.

Regulatory pressures for improvements in the scientific understanding and quality of drug product is also leading to an evolution in analytical workflows. "We are seeing increasing guidelines focused on analytical development, such as a [Brazilian Health Regulatory Agency] ANVISA guideline on conducting forced degradation studies that is very demanding," Carr observes.

Automation improves sample prep

Some of the most important advances in sample preparation tools include increasing application of automation and robotics. Quality-by-design (QbD) approaches to analytical testing can often lead to multiple sampling and testing to achieve a more accurate assessment of the total batch rather than testing one or two samples per batch.

An example given by Carr is stratified sampling for solid oral dosage forms whereby samples are taken at approximately 20 time points during tablet compression or capsule filling and tested for drug content, and three units from each time point are tested. "In circumstances such as this one, there are huge benefits in having automation available in the lab for the preparation of the analytical samples from the 60 individual tablets that require testing," he explains.

Automation of sample preparation for low throughput methods is also critical to improve turn-around times to support process development activities, adds Katiyar. In general, he notes that automation of all in-process methods for biologics—including size-exclusion chromatography, CGE, iCIEF, n-Glycan content and residual host-cell protein, DNA, and Protein A—to support process development activities is crucial for meeting fast-to-first-in-human (FIH) trials/quick-

to-clinic timelines. "In addition," he says, "late-stage programmes with QbD filings are also exploring better turnaround times to support expanding pipelines."

In the field of biologics sample preparation, Process Development Scientific Director Jill Crouse-Zeineddini at Amgen sees acoustic droplet ejection for potency assays as an important advance. Acoustic droplet ejection uses acoustic energy instead of tips to transfer a fixed amount of liquid sample from a source to destination plates freely with excellent accuracy and precision, she explains. "The significance of this technology resides in its superb dispensing performance at a very low sample volume. This technology performs direct dilutions instead of serial dilutions and prepares each dose independently, improving assay precision and throughput," Crouse-Zeineddini observes.

Robust aseptic sampling and automated sample preparation for the purification, desalting, and digestion of protein samples, meanwhile, enables many different product quality analyses. "This technology not only significantly improves operational efficiency, but also eliminates potential contamination and mistakes during manual sampling handling," states Gang Xue, process development scientific director at Amgen.

Another important point, according to Carr, concerns the reliability of the sample preparation procedure. "This issue is not a new one, but it is becoming more apparent as we apply QbD approaches to our analytical procedures. While the greatest emphasis has been applied to chromatographic parameters, we now realize that the sample preparation stage is at least as important and also needs to be developed using QbD," he comments.

For Lee, a specific technology development that has improved sample preparation is once again material identification by Raman spectroscopy for small-molecule drug substances. "Using this technique simplifies sample preparation, because it allows identification of compounds without any physical contact with the sample. Depending on the container material, it is even possible to acquire Raman data through the container without the need to withdraw a sample," she says.

More developments on the horizon

Such capability for biologic drug substances has yet to be developed, however, and simplified identity methods to support release and establishing post-shipment identity of bulk drug substance are still required, according to Katiyar. Currently, peptide mapping and binding ELISA are used as identity methods, but they have long turnaround times. Raman spectroscopy has been evaluated for biologics, but it has not yet been adopted by the industry for release of drug substances and drug products. "Simplification using scan-based methods with better specificity and faster turnaround times would be highly beneficial for biopharmaceuticals," he says.

When integrated with analytical instruments, aseptic sampling and automated sample preparation has the potential to move in-process and product release testing from offline QC labs to the manufacturing floor, either in-line or online, according to Xue. In addition to enabling real-time monitoring of not only cell growth, but also the critical quality attributes of therapeutic proteins themselves, the technology is beneficial for providing much more granular insights into the conventional batch process and products in-flight, he notes. "More importantly," Xue states, "it could in the future be crucial for lot definition, process variation detection, and material segregation as required for continuous bioprocessing."

For small-molecules, Lee would like to see the widespread adoption of x-ray fluorescence (XRF) for metals testing because it would also reduce turnaround times. "XRF is a powerful method for detecting metals used as catalysts in API manufacturing. Compared to inductively coupled plasma-MS, XRF does not require sample dissolution and digestion, facilitating easier sample preparation and faster turnaround times," she explains.

Carr, meanwhile, expects to see increasing use of LC–MS for routine analytical testing. "This technology is widely applied in chemical drug development labs for various purposes and is also used for biopharmaceutical analytical testing, but less for release testing of products and for testing stability samples. The technology has advanced considerably over recent years, and while these instruments were previously only applied in R&D, they have now become highly suitable for use in routine testing labs," he remarks.

Short timelines create challenges

There are a number of challenges to the adoption of advances in analytical techniques, some of which vary according to the development phase. Adhering to compressed programme timelines is the key challenge in getting advances in method adoption for early stage development, according to Katiyar. "Fast-to-FIH/quick-to-clinic programme timelines have been introduced in almost every pharmaceutical organization to provide clinical material for Phase I studies, and these timelines have shrunk from 18 months to less than 12 months during the past five years," he says.

The shorter timelines are met by relying on platform approaches developed based on knowledge generated over years with multiple molecules. "For new molecules that fit the platform methods, there is no scientific justification to explore new technologies," Katiyar states. When working in a lab that is operating in a high-efficiency environment, there is often resistance to the introduction

of new methods and approaches due to concerns about meeting delivery targets, agrees Carr.

Once programmes move to late-phase development, organizations are hesitant to introduce any change in the control strategy unless it is absolutely needed. This reluctance is particularly strong if a filing has been made to a regulatory agency and/or if significant data have been collected using the older technique, according to Spivey.

"To be adopted for measuring product quality measurement, the performance of new analytical methods must be equivalent to or better than the methods they replace, and there must be clear evidence that they are reliable and robust across a wide range of operating spaces," states John Harrahy, director of process development in pivotal attribute sciences with Amgen. The adoption of new technology in the middle of a programme, adds Katiyar, requires significant effort to develop the new method, perform bridging studies, requalify the method, perform technology transfer (if outsourced), perform retrospective testing, and define new specifications. Bridging studies cost the sponsor additional money and time, and there is always the risk that a bridging study may show that the methods or techniques are not comparable, adds Spivey.

There is also often a reluctance on the part of drug companies to be the first to make a submission to FDA with a new technique due to the possibility of the validity of the technique being questioned, Spivey notes. "They don't want the burden of having to defend the technique to FDA or other regulatory agencies," she says. There can be some risk with introducing new technologies that have had limited regulatory exposure, adds Harrahy, particularly considering the different regulatory expectations and change control requirements from different regulatory authorities worldwide.

"With that said," Harrahy comments, "evaluating innovative technologies is a vital component to ensuring product quality and value to patients, and the ultimate risk of not evaluating new technologies greatly outweighs remaining stagnant."

The ideal solution, Katiya argues, is to explore new technologies as part of improvement initiatives without associating them with any programmes. This approach provides the flexibility to explore new technologies without putting the programme timelines at risk. "Once proof of concept is established and the method is ready to be adopted, a platform approach can be used to implement the new technology," he comments.

Senior leadership in large organizations, according to Katiya, must provide guidance to their teams to push innovation without risking programme timelines. In addition, it is also important to apply thorough training practices to ensure that scientists really understand the new approaches, says Carr. Continuity of data must also be addressed. "Trend analysis is a widely used tool for monitoring pharmaceutical product quality, and the introduction of new and 'better' methods may be perceived to interfere with this trending process," Carr observes, even though it is more important to apply continuous improvement and accept possible breaks in trends.

Ways to facilitate adoption of new analytical technology

In addition to evaluating new analytical methods separately from specific drug development programmes, there are several other strategies that can be used to facilitate the adoption of advances in analytical techniques.

The best strategy for adopting a new analytical method in a quality setting, according to Harrahy, is to start with the end in mind. Does the proposed method fit the analytical target profile? Is the method sufficiently capable for the product or products that it will measure? Does the methodology require modification to the available GMP/QC environment?

"The robustness, reliability, and value of introducing any new method must be clearly demonstrated, which is often best accomplished by taking a staged approach: determining the method operable design space in a development laboratory, piloting the method in a development/phase-appropriate setting to monitor 'real-world' method capability, performing bridging studies vs. the older method, staging its implementation in QC, and continuously monitoring method performance," he says. In addition, for regulatory acceptance of novel technologies, early partnered engagement with health authorities is strongly recommended.

The most important strategy, agrees Spivey, is to provide ample data demonstrating that new methods are reliable and robust and that there is little or no risk to implementing the technique in a regulated environment. Advances that offer significant advantage over corresponding currently accepted techniques will also have greater likelihood for acceptance. However, Spivey stresses that the advantage would need to be significant enough to be worth the time and money needed for it to be implemented. "Ideally," she says, "the owner of the technique would perform some preliminary legwork with the regulatory agencies demonstrating the capabilities of the technique. The sponsor would then have some assurance that the agencies would accept their data and make it a less risky approach for them."

Another approach, depending on the nature of the old and new/improved methods, is to run both in parallel for a period of time in order to develop an understanding of how their performance and the resulting data compare, Carr suggests.

For Lee, the key to new analytical method adoption is the sharing of use cases between pharmaceutical companies combined with the publication of white papers and communication with regulatory authorities. Katiyar agrees that

sharing knowledge and gaining the feedback of peers and regulatory authorities in a timely manner is essential. "Peer-reviewed publications, conference presentations, and Biophorum Operations Group-like forums are the best places to share information and exchange ideas to improve and adopt new technologies on a global scale," he comments.

All stakeholders must collaborate

That information sharing should occur between all stakeholders, including contract research, development and manufacturing organization, testing laboratories, biopharmaceutical companies, regulatory authorities, and instrument/equipment vendors.

"Innovators and service providers need to be open to new ideas and be willing to invest the time and money to implement new techniques. Service providers also, rather than waiting for clients to request a technique before investing in it, should advocate for the use of new methods with their clients," Spivey asserts.

In addition, Katiyar believes innovator companies working with service providers should form an external working group to share new methods and technology to eliminate knowledge gaps caused during technology transfer of methods. "Most of the time," he remarks, "innovator companies are not willing to share new methods and technologies and thus delay the adoption of new technologies throughout the pharmaceutical industry."

Regulators also need to be open to new ideas and willing to work with pharmaceutical companies to ensure that new methods and techniques are acceptable for use in a regulated environment, according to Spivey. It is important for pharma companies and regulatory authorities to remember they have a common goal in identifying new methods and technologies for monitoring and quantifying critical quality attributes that may impact the safety and efficacy of the molecule throughout the lifecycle of the programme, adds Katiyar. He points to MAMs as an example where health authorities have accepted data packages consisting of results obtained using conventional approaches supplemented by those obtained using MS-based approaches.

Instrument/equipment vendors, meanwhile, should be prepared to demonstrate that a new technique is sufficiently better than the currently accepted technique to be worth investing in and worth any potential regulatory risks, asserts Spivey. The dilemma here, according to Carr, is how stakeholders all link together.

"If a new analytical technology comes up, it will not be accepted by industry/regulators unless the equipment that is required to use it becomes widely available. Maintenance, qualification, and repair services must also be widely available and reliable. Typically, however, a vendor will not set establish this level of availability unless there is a level of confidence that sales targets will be achieved. I think that this is the area where conferences, exhibitions, and publications provide a really valuable platform to get the information from innovators and suppliers circulated to end users," he says. **PTE**

The Search for Transparency in Excipient Sourcing

Researching excipient grades and sources, as well as screening suppliers and materials, form the basis of programmes to mitigate risk.

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

Although not pharmacologically active, excipients have a direct impact on the performance of formulated drugs, and their quality and purity are equally important to assuring drug product safety. Because excipients are seldom pure compounds, consistent composition is of utmost importance, asserts Irwin Silverstein, president of IBS Consulting in Quality. Conformance to good manufacturing practices (GMP) is also important so that the customer can rely on the excipient manufacturer's certificate of analysis (CoA).

Pharmaceutical excipients are typically multifunctional chemistries not exclusively designed and formulized for the pharmaceutical industry. As a result, these molecules often originate from other applications and industries and are later utilized within the pharmaceutical industry, according to Jessica Cansler, a senior quality management specialist with BASF. "The initial manufacturing and quality standards of these molecules were focused more to the requirements of the original application areas and industries, not to the manufacturing and quality requirements of the pharma industry," she says.

It is incumbent upon pharmaceutical manufacturers to understand the full excipient supply chain and have in-depth knowledge about selected suppliers. Recognizing that risk cannot be avoided but must be mitigated is equally important.

Many excipient sourcing challenges

The challenges to excipient sourcing vary according to the type of supplier from which the manufacturer is purchasing the material. "Lack of transparency about manufacturing locations and the entire excipient supply chain can provide challenges," notes Fernanda Onofre, technical service manager for pharma solutions at DuPont Nutrition & Bioscience.

One of the biggest challenges, perhaps, is that many industrial-scale manufacturers make products in multi-application plants, and the volumes produced for the pharma industry are typically only a fraction of the total production, making it difficult to fulfil pharmaceutical industry expectations (GMP, quality, etc.), according to Cansler.

It can also be challenging to trace back the source of the original materials and understand the quality level of the ingredients, as many are 'tested up' to monograph standards rather than produced under the appropriate guidelines, she notes. In some cases, it can be difficult for drug manufacturers to find an appropriate excipient manufacturer fulfilling pharma quality expectations, particularly for legacy products; sometimes drug manufacturers must make compromises and accept what is available on the market.

When the excipient is shipped directly from the excipient manufacturer to the drug manufacturer, there should be no misunderstanding as to the source, according to Silverstein. "However," he observes, "purchasing through a distributer, broker, or trader may raise a challenge, especially where the excipient has been packaged from bulk or repackaged from discrete packages. Tracing the excipient back to the source relies on having confidence in the packager or repackager and receiving the excipient manufacturer's certificate of analysis."

Issues in the past, according to Chris Moreton, principal with FinnBrit Consulting, have related to the sourcing by a distributor of excipients from multiple manufacturers without notifying the customer, which had only validated one source of supply, as well as the switching of plant species from which excipient starting materials have been derived, impacting excipient performance in the manufacture of the finished drug product. Distributors, Moreton says, must inform their customers of the manufacturing sites for every delivery of an excipient.

The excipient manufacturing process can also provide challenges. "This information is often proprietary, yet drug manufacturers need to understand the raw materials and processes that can impart variability in excipients," Onofre notes. Regardless of the source, therefore, pharma manufacturers must be willing to invest

the time and effort in establishing monitoring systems based on the use of advanced spectroscopic methods, notably for plant-derived excipients, in order to detect unexpected changes in impurity profiles from a single supplier or variations in profiles from different suppliers of the same excipient, according to Moreton.

Pharma companies should also be sure to conduct supplier qualifications to ascertain the necessary details about excipient suppliers, a process that can provide challenges as well, according to Onofre. "Thorough qualification requires an on-site audit of the excipient supplier, either by the pharma company themselves or by a trusted third party," she says.

Know the supplier

The first step, Onofre adds, is for pharma companies to fully engage with their excipient suppliers, asking for clarity on the excipient's entire lifecycle. "Even in the instances where information may be proprietary, much can be clarified after a discussion with the supplier," she comments. Detailed discussions under a confidential disclosure agreement (CDA) can also help in understanding excipient variability and how that can affect the formulation and process. "Such discussions aid in excipient risk management and the formulation of robust drug products," Onofre states.

Drug makers should also insist that the manufacturing site of the excipient be identified on each lot of the excipient received at the product manufacturing site, Moreton asserts. The International Pharmaceutical Excipients Council (IPEC) CoA guide (1) establishes that the site of manufacture should be disclosed on this document, according to Silverstein. "Ideally, the batch numbering system of the excipient should be site specific, such that a change in the format of the batch number would act as a check for the site of origin," Moreton adds. Any change in sourcing of the excipient starting material sourcing should also be included as part of the change notification requirements in the quality agreement or in a commercial agreement, with change notification completed per the IPEC significant change guide (2).

On-site audits conducted by the pharma company itself or via a third party, such as EXCiPACT— an international consortium of excipient, chemical, and quality organizations— should be conducted to gather as much information as possible about the supplier and the excipient, Onofre says. If a third-party has been used for the audit, Silverstein adds that it is important to obtain a copy of the audit report to help assure the validity of the audit conclusions concerning production of an excipient in conformance to GMP.

To verify the source of the delivered excipient, whether in the original manufacturer's container, packaged from bulk, or repackaged, Silverstein comments that the user should verify the authenticity of the excipient label, package, and tamper-evident seal.

Overall, the best approach is to source excipients from excipient manufacturers that clearly commit themselves to the pharmaceutical market and the expectations of the pharmaceutical industry, Cansler asserts. "Usually these excipient manufacturers are appropriately certified to pharmaceutical industry quality standards (e.g., NSF/IPEC/ANSI 363 and/or EXCiPACT)," she says.

Pay attention to red flags

When considering different excipient suppliers, there are some warning signs that could indicate potential issues for pharmaceutical manufacturers. For Moreton, a top red flag is not naming the site of manufacture because that precludes physical audits. "Reliance on the distributer's paper audits or third-party audits for and on behalf of the distributer—and in which the excipient user has no control and is not consulted ahead of time—should not be acceptable," he says.

The claim that an excipient will meet the *United States Pharmacopeia* (*USP*) monograph when tested is a key warning sign for Silverstein. "This language may indicate that the excipient was not produced under 'appropriate' GMP as required by *USP*. Refusal to share non-compendial test methods is another red flag; a good supplier wants to facilitate the proper testing of their excipient, he says.

Pharma manufacturers should also be wary of excipient suppliers that are not aware of current issues, concerns, and trends in the industry they serve, or have to custom-make standard documents, according to Cansler.

Suppliers that are not known in the pharma industry or only have a small presence, as well as those that are unable to provide the required qualification documentation, historical manufacturing trend data under CDA, and support manufacturing location audits raise concerns as well, adds Onofre. Finally, Moreton points to suppliers that are unwilling to enter into quality/change notification agreements as raising red flags.

On the flip side, reliable excipient suppliers exhibit a number of recognizable attributes that should be sought. Most importantly, reliable excipient suppliers are open, albeit under confidential disclosure agreements, according to Moreton. They will allow either customer audits or certification to excipient GMP by a recognized accreditation body or certification scheme, adds Silverstein.

These excipient suppliers recognize the importance of the pharma market to their business profitability and participate in appropriate trade associations and meetings. They also have deep knowledge of their products and can provide technical support for their use in drug formulations and provide products with reliable excipient quality, consistent composition, documentation in order, and an established quality management system at the excipient manufacturing site that complies with pharma excipient GMP requirements and expectations, according to Cansler.

Selection essentials

In addition to an initial onsite audit, pharma manufacturers should closely

evaluate several aspects of excipient supplier performance during the selection process. Identification of the GMPs to which the supplier intends to conform is paramount, according to Silverstein. Standard quality, regulatory, and technical documentation for the excipient should be readily available and complete, coupled with a point of contact to ask questions, Cansler observes. Evidence of the supply chain, quality, and services being offered by the supplier should also be provided, along with in-depth information on the manufacturing locations, notes Onofre.

Access to multiple excipient samples from different campaigns and different raw material lots for qualification is also important, adds Cansler. Excipient suppliers should also sign quality/change notification agreements as part of the overall supply or quality agreement, says Moreton.

Finally, once a supply agreement is in place, Moreton and Silverstein both note that an effective monitoring programme at the excipient user site should be implemented to assess the quality and technical characteristics of the excipient.

Excipient grades matter, too

Excipient grades are typically differentiated based on a certain physical characteristic (e.g., particle size, molecular weight, degree of substitution, or viscosity). A change in grade means a change in that physical characteristic. For some formulations, a change in grade may not impact product performance, but for others it could be detrimental to the finished product, according to Moreton.

"Different grades allow for flexibility in formulation and process design to address various technical challenges in other raw materials or processes used to manufacture the final dosage form," Onofre explains. She adds that different grades are also critical for optimizing the right process, which can help in cost-saving efforts in areas from wet granulation to direct compression.

If a specified excipient grade is important to the quality attributes of the drug product, then the formulator should demonstrate in what manner the grade is important, adds Silverstein. "In this case, the homogeneity of the excipient lot for that attribute becomes critical to drug product quality, and the excipient manufacturer should be asked about homogeneity within each lot," he says. In addition, a change in grade most likely would necessitate a revalidation and a notification under the scale-up and post-approval change rules, Moreton comments.

Choosing the right excipient grade used to be at the personal preference of the formulator, but with quality by design widely implemented today, properly designed and executed design of experiments studies give support to the choice of excipient grade, according to Moreton.

In addition to information provided by excipient suppliers, pharma companies may also conduct grade-differentiating testing when establishing excipient specifications. Talking to the excipient technical experts and sharing the intended use for the excipient and the issues to be addressed in the designed formulation can also help in grade selection, according to Onofre.

Assuring receipt of the right excipient grade starts at a minimum with identification testing and testing against the CoA/monograph listed tests and parameters, according to Cansler. Services such as infrared scanning flaps/windows in packages, whenever possible, can help reassure the customer about the accuracy of the product and reliability of the supply, adds Onofre.

It is also important to confirm product labelling and product documentation and compare them with the purchase request, Cansler observes. "The excipient specification at the drug maker should clearly link to the excipient supplier's specification for the grade, and the purchase order should also clearly delineate the grade as it will appear on the excipient package label and CoA," Silverstein explains. In some cases, he notes that it may also be necessary to discuss with the excipient manufacturer the recommended test method for the parameter that designates the grade and that the accuracy and precision are sufficient to confirm the grade.

Managing risk is key

The most important concept to grasp when dealing with the excipient supply is "Caveat emptor: let the buyer beware!" asserts Moreton. "There are no certainties, and there is always a risk that something may go wrong. However, the risk can be reduced to an acceptable level, but only by acquiring as much knowledge as possible about the excipient and using a combination of site and supply chain auditing, quality/change notification agreements, and an effective monitoring programme for all excipient deliveries to the site of use," he states.

In addition to risks associated with manufacturing processes for excipients, there is risk associated with the distribution and supply chain of excipients globally, notes Cansler. She points to risks associated with the intermediate storage and transportation of excipients. "Trending topics including fraud and adulteration within the supply chain are of serious concern. In addition to raw material qualification, appropriately established supply-chain traceability and supply-chain security processes should be in place," she observes.

The IPEC excipient qualification guide (3), according to Onofre, provides details on recommendations for building a robust supplier qualification process. "Building customer-supplier relationships that are transparent and provide robust information-sharing opportunities is the foundation for success," she concludes.

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Improving Solubility of Cannabinoids

Lipid-based solutions offer a natural approach to improving the solubility of cannabinoid-derived products.

Felicity Thomas

In recent years, marketing authorizations of cannabinoid-based pharmaceuticals (namely nabiximols [Sativex] in Europe and plant-derived cannabidiol [Epidiolex] in both Europe and the United States) have been issued globally, although legal and regulatory concerns around cannabis-based products are still apparent (1). As more cannabinoid derivatives are currently in development, there is a drive for developers and formulators to address the challenges these ingredients pose.

To explore the common issues that can be encountered with cannabinoids during formulation and development and the potential solutions that are available to overcome these challenges in more detail, *Pharmaceutical Technology Europe* spoke with Alyn McNaughton, technical director, Lonza Pharma & Biotech, and David Fulper, director, Technology Support, Softgel and Oral Technologies, Catalent.

Ingredient challenges

PTE: What are the specific challenges facing formulators and developers when approaching cannabinoid-derived products?

McNaughton (Lonza): Cannabinoids, generally, have a very low solubility in water and are highly lipophilic. As an example, cannabidiol (CBD) has a solubility of only 0.7 µg/mL (2). Consequently, these materials cannot readily be absorbed orally and, therefore, a large quantity is required to have a medicinal effect. The bioavailability is also significantly affected by dietary fat, which can lead to variability. Alternative routes of administration, such as smoking, present additional challenges, such as potentially producing toxic by-products. These other routes of administration also do not have a significantly better bioavailability and the variability is generally even higher than the oral route.

Fulper (Catalent): Stability and first-pass metabolism are probably the two biggest technical challenges. Cannabinoids tend to be oxidatively sensitive, which can be affected by both the formulation and manufacturing process. Tetrahydrocannabinol (THC), and probably CBD, undergo extensive first-pass metabolism, which can be particularly challenging for oral formulations.

An additional challenge is the desire to use extract or 'broad spectrum' material that contains multiple components. The variability of these extracts makes it difficult to establish API specifications, develop analytical methods, and even to interpret clinical data. Then there is the political/regulatory challenge for anything related to cannabis, which can often complicate the process.

Synthetic versus plant

PTE: Are there any inherent differences between plant-based cannabinoids or synthesized ones that may impact drug development and formulation?

Fulper (Catalent): Molecularly, there are no differences in plant-based cannabinoids and synthetic cannabinoids. As with any raw material supply, there is always the question of purity and related substances, which could differ between the two. The question more often seems to stem from the desire to include other components of plant-based material for a therapeutic effect. The validity of this approach can only be determined through application of adequate science.

McNaughton (Lonza): The source, synthesized or extracted, is becoming less relevant to the drug development cycle for cannabinoids as regulators apply pharmaceutical practices. Combinations of different cannabinoids become costly and challenging to get approved due to the permutations of safety and clinical trials required with variations in ratios of these materials required to prove efficacy and safety. From either source, single, pure cannabinoids present the most straightforward route for approval.

While some evidence exists that there are benefits in certain cases for mixtures of cannabinoids, the approval path for this approach is prohibitive and it is likely, at least in the near future, that development of a single, pure cannabinoid will be the preferred route for a pharmaceutical. In other sectors,

such as the nutraceutical market, it is likely that some mixtures will still be developed. These mixtures may return to pharmaceutical development areas once the properties of the cannabinoids are more fully characterized individually.

The nature of some cannabinoids to be psychoactive, and consequently by their presence turn any materials into a controlled drug substance, also presents a challenge, mainly for the extracted products. However, extracted materials or those synthesized at a late stage from extracted starting materials still appear to be cheaper than those fully synthesized, at least for the moment, but this is likely to change as the chemistry of synthesis becomes more commonly used.

Currently available solutions

PTE: Could you highlight the currently available solutions that can help formulators overcome the solubility and bioavailability challenges associated with cannabinoids?

McNaughton (Lonza): Although other solubility-based bioavailability enhancement approaches, such as solid dispersion, can present some improvement, lipid formulations are the natural approach for molecules with high lipophilicity and low water solubility. Solubility of the cannabinoid in the lipid, and associated excipients, creates an overall solubilized formulation that avoids solid state limitations for absorption.

Fulper (Catalent): Cannabinoids tend to fall into Developability Classification System (DCS) Class IIb (solubility rate-limited absorption), and formulation technologies that improve intrinsic solubility, such as lipid-based systems or solid dispersion systems, may help to address the solubility aspect of poor bioavailability; however, if the compound suffers from high first-pass metabolism overall, bioavailability will remain low. Increasing the dose can sometimes saturate the enzymes involved, but this approach can result in an increase in undesired side effects.

The log P for these compounds tend to be in a range conducive to lymphatic absorption. Using lipid formulations that contain long-chain unsaturated fatty acid components help promote chylomicron production, which are then lymphatically transported into the blood stream and bypass first-pass liver metabolism. The degree to which a specific cannabinoid is incorporated into chylomicrons remains an open and important question.

Benefits of lipid-based approaches

PTE: Why are lipid-based solutions particularly beneficial for cannabinoid-derived products?

Fulper (Catalent): Cannabinoids are generally oxidatively unstable. Softgel delivery platforms used to deliver lipid-based formulations are excellent oxygen barriers, and the manufacturing process to make softgels is conducive to protection from exposure to air. Cannabinoids generally have good solubility in triglyceride lipid bases, allowing for easy solubilization in lipid formulations. These formulations, in turn, are readily digested by the body into mixed micelle systems that are readily absorbed into the enterocytes, the intestinal absorptive cells. Cannabinoids can suffer from first-pass liver metabolism. Lipid formulations that promote the formation of chylomicrons offer a path to bypass first-pass liver metabolism.

McNaughton (Lonza): Along with the generic potential for lipids to overcome the solid-state limitations for absorption and present the cannabinoids in a solubilized form for intestinal absorption, lipids also present further benefits for highly lipophilic molecules. A secondary contribution to the low bioavailability is the potential for many of these molecules to suffer from first-pass metabolism, where the drug is metabolized, usually by the liver, reducing the quantity reaching systemic circulation. For drugs with a LogD > 4.7 and solubility of >50 mg/g in LCT (long-chain triglycerides) (3), appropriate formulation can promote intestinal lymphatic absorption, over the normal hepatic portal system, undergoing transport directly to the systemic circulation and avoiding first passing through the liver.

Lipid selection

PTE: Could you provide any advice on lipid selection for cannabinoid formulations?

McNaughton (Lonza): The selection of excipients comes down to the expertise of the formulator, though there are some general approaches that can be applied. The incorporation of an LCT is preferable, if the cannabinoid is soluble enough in the LCT and the LogD > 4.7. Formulations where LCT, or other triglyceride, is the only excipient are very likely to have large patient and dietary variability. The formulator, therefore, needs to refine the formulation with appropriate excipients, such as surfactants and co-solvents, to allow an emulsion, or preferably a micro-emulsion, to be formed. Ideally, the cannabinoid should also be as soluble in the additional excipients. The closer the formulation is to a micro-emulsion, the less likely it is to have patient variability. As well as being able to form and sustain the emulsion through dilution in the gastric system, it is also critical that the solubilization survives the change into the intestinal system and that the digested species of the lipid formulation also continue to solubilize the cannabinoid long enough for absorption to take place.

Fulper (Catalent): Lipid selection should always include an assessment of their impact on stability. Many lipids themselves can undergo oxidation. If a less stable lipid is chosen, for instance to target lymphatic absorption, then the source of that lipid should be evaluated for incoming quality. When selecting a lipid to promote lymphatic uptake, long-chain fatty acids are preferred, and unsaturation is preferred to saturated lipids. Solubility should be good in most triglyceride-based excipients. *In-vitro* digestive studies can be used to evaluate the relative ability of the formulation to maintain solubility during digestion.

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Quantitative Rupture Testing of Soft Gelatin Capsules: Understanding Aberrant Results

Donald A. Johnson

This paper discusses what causes cross-linking, how cross-linking is addressed with addition of enzymes, and consideration for occasional high results that can be obtained during release testing.

Soft gelatin (softgel) capsules can be a means of achieving bioavailability of highly lipophilic drugs that are practically water insoluble. The API is generally dissolved in edible oil. Typical oils used in softgel capsules are medium-chain triglycerides such as Miglyol (IOI Oleochemical) and mono- or di-glycerides such as Capmul (Abitec). For softgel capsules containing lipophilic drugs, the Division of Bioequivalence (DBE), in the Office of Generic Drugs, Center for Drug Evaluation and Research, in the US Food and Drug Administration (FDA), will ask applicants to submit a "quantitative rupture" *in-vitro* drug release test to measure the drug released in the dissolution medium after the capsule shell ruptures (1). The method will typically use a dissolution apparatus 1 (basket) or apparatus 2 (paddle) described in *United States Pharmacopeia* (*USP*) <711> Dissolution. In this paper, quantitative rupture testing and dissolution testing will mean the same thing for softgel capsules. Even when the drug is already completely dissolved in the capsule fill, the capsule shell still needs to dissolve for drug release.

A suitable surfactant at an appropriate concentration in the aqueous dissolution medium is a key element for quantitative rupture testing of these softgel formulations. Developing a quantitative rupture test for capsules containing lipophilic drugs formulated in oils presents many challenges. Cross-linking of the capsule shell is the most common challenge. With time, cross-linking will increase, and drug release values may fall below established specifications. Tier 2 release testing with digestive enzymes is an acceptable solution to cross-linking of gelatin capsules (2). During Tier 2 testing of softgel capsules that have cross-linked, an occasional aberrant high value for a capsule may be found. This could be, at times, more than 200% of label claim. This paper discusses what causes cross-linking, how cross-linking is addressed with addition of enzymes, and consideration for the occasional high results that can be obtained during release testing.

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Table I. Physicochemical properties of APIs in softgel products. MW is molecular weight; P is partition coefficient.

Compound	Description	MW (g/mol)	Aqueous Solubility	Log P
Paricalcitol (4)	White, crystalline powder	416.646	$1.2X10^{-6}$ mg/mL	4.5–7.4
Dutasteride (5)	Solid	528.539	$9.08x10^{-4}$ mg/mL	6.8
Ergocalciferol (6)	Odourless white crystals	396.659	0.05 mg/mL	7.3–10.44

Physicochemical properties of drugs in softgel products

There are two important properties that must be taken into account for getting an oral drug absorbed through the walls of the gastrointestinal tract and into the body: solubility and permeability. If a drug is insoluble in aqueous media, the drug can have very limited bioavailability because there is not a significant driving force for partitioning of the drug from the aqueous environment of the intestinal lumen into the intestinal membrane. On the other hand, if a drug is too soluble in aqueous media, it may not want to partition into the lipid layers of the intestinal membrane. The solubility/permeability trade-off is the basis for the Biopharmaceutics Classification System (BCS) (3).

For drugs that are highly lipophilic, a softgel capsule product can be a useful dosage form for increasing bioavailability of the drug. For the purpose of this paper, the softgel products discussed are those in which the capsule fill contains the drug completely dissolved in oil. The drug will have a high concentration in the oil phase relative to an aqueous medium as indicated by the partition coefficients of the three APIs shown in **Table I**. Without the oil phase, the concentration of the drug in the aqueous environment of the intestinal lumen is too low to provide a driving force for intestinal membrane permeability.

With softgel products, the drug is already dissolved in the oil fill of the product and drug dissolution is not a physical process that occurs during rupture testing of softgel capsules. Many still refer to release of drug form softgel products as dissolution testing because the quantitative rupture testing generally follows *USP* <711>. It is the capsule shell that dissolves in order for the drug to be released.

Table I shows typical physicochemical properties of three softgel capsule products that have the drug dissolved in an oil fill.

It is seen in **Table I** that the aqueous solubilities of the three drugs are very low, ranging from nanograms per millilitre to micrograms per millilitre. The solubilities in lipid media relative to their solubilities in aqueous media are high for the three drugs, as indicated by their high partition coefficients, P. The partition coefficient measures the concentration ratio of a drug at equilibrium in a lipid medium and aqueous medium, where the two media are immiscible but in contact. Partition coefficients are commonly determined with octanol and water. In **Table I**, the partition coefficients show that these drugs have solubility in lipid media that range from approximately 100,000 to more than 10 million times the solubility in aqueous media. Drugs with large partition coefficients would have high permeabilities across the intestinal membrane, thus drug dissolved in an oil fill of a softgel would be an ideal dosage form for drugs with similar properties to those in **Table I**.

Table II. Low result (vessel 6) due to cross-linking.

% drug released	
Vessel #	**45 min**
1	98
2	91
3	95
4	96
5	62
6	**62**
Mean	89
Min	62
Max	98
%RSD	15.1

Table III. : High result (vessel 3) due to cross-linking.

% drug released	
Vessel #	**45 min**
1	101
2	92
3	**163**
4	98
5	97
6	95
Mean	108
Min	92
Max	163
%RSD	25.3

Impact of cross-linking on drug release of softgel products

Gelatin is derived from the partial hydrolysis of collagen. Gelatin crosslinking occurs due to chemical reactions between the peptide chains of gelatin. Once formed in a softgel capsule shell, the capsule shell will only rupture in the presence of proteolytic enzymes if the cross-linking is significant.

Cross-linking is a common problem encountered in the dissolution of gelatin capsules and is most commonly seen during stability testing. Low and incomplete dissolution may be observed while performing *in-vitro* release testing, as the capsule shell may dissolve slowly and incompletely, delaying full release beyond the specified sampling time point of the test method. If severe cross-linking occurs, bioavailability issues may also arise.

Cross-linking may not occur evenly across a container of drug product. **Tables II and III** show typical results that may be seen during release testing. In both cases, only one out of six capsules tested has appreciable cross-linking. **Table II** shows a case where a single low value is obtained. This result is more common in cases where no enzyme is added to the dissolution medium (Tier 1 testing as explained in the following section). In **Table III**, an aberrant high result is obtained in one capsule. This result is commonly seen in testing with enzymes (Tier 2 testing).

Table IV. Dissolution criteria for two-tier dissolution testing.

	Criteria	
Stage	**Tier 1 (without enzymes)**	**Tier 2 (with enzymes)**
Stage 1	Test six capsules Each capsule is Q+5% If criteria not met go to stage 2	Test six capsules Each capsule is Q+5% If criteria not met go to stage 2
Stage 2	Test additional six capsules The average of the 12 capsules must not be less than Q No capsule should be less than Q-15% If criteria not met go to stage 3	Test additional six capsules The average of the 12 capsules must not be less than Q No capsule should be less than Q-15% If criteria not met go to stage 3
Stage 3	Test additional 12 capsules Average of 24 capsules must not be less than Q No more than two capsules are less than Q-15% No capsule is less than Q-25% If criteria not meth go to Tier 2	Test additional 12 capsules Average of 24 capsules must not be less than Q No more than two capsules are less than Q-15% No capsule is less than Q-25%

Current *USP* approach to cross-linking

When a softgel product fails dissolution acceptance criteria due to cross-linking, *USP* allows the use of enzymes in the dissolution medium and allows for two-tier dissolution testing. In Tier 2, pepsin is added to acidic or water dissolution medium to achieve an activity of 750,000 units or less per liter. Pancreatin *USP* is added to a dissolution medium at or above pH 6.8 to achieve a protease activity of not more than 1750 units per liter.

The two-tier dissolution testing is described in *USP* <711>. Dissolution criteria for dissolution testing are expressed with a time point and a Q value. For example, a dissolution specification may be 75% (Q) at 45 minutes. **Table IV** shows the criteria for the two tiers in *USP* <711>.

In Tier 1, the test is performed with the normal dissolution medium of the test method. If the product fails Stage 3 criteria at any stage in Tier 1, testing progresses directly to Tier 2, where enzymes are added to the dissolution medium. For example, if cross-linking is significant, failure to meet Stage 3 criteria in Tier 1 may occur at Stage 1 testing. In this case, there is no need to perform Tier 1 stages 2 and 3. Testing should proceed directly to Tier 2 testing. Most commonly, Tier 2 criteria are met at Stage 1 of Tier 2. Any failure to meet Tier 2 criteria at Tier 2 Stage 3 is a failure of the dissolution specification and indicates a problem for the product.

Pretreatment of softgel capsules when using enzymes and surfactants

During quantitative rupture testing of softgel capsules that contain an oil fill, a surfactant must be added to the dissolution medium in order to solubilize the oil once it is released from the capsule shell. Otherwise, when the capsule shell ruptures, the oil droplets will be released and simply float to the surface of the dissolution medium. The amount of surfactant added to the dissolution medium must be sufficient to form micelles. The lipophilic core of the micelle is able to take up the oil-containing drug released from the capsule upon rupture.

Although surfactants are a necessary component in quantitative rupture testing, they are known to inhibit the activity of enzymes used in the dissolution medium when Tier 2 testing is performed.

To prevent inactivation of the enzymes in Tier 2 testing, pretreatment of the cross-linked gelatin capsules with the medium containing the enzyme but not the surfactant must be performed. Pretreatment is performed by adding the enzyme to a portion of the dissolution medium without the surfactant and stirring the medium in the dissolution vessel for a short period of time, usually not more than 15 minutes. After this period, the remaining portion of the dissolution medium containing the surfactant is added to the dissolution vessel. After addition of the remaining dissolution medium, the final concentration of the surfactant in the vessel and the final dissolution medium volume will be the same as specified in the test method. The pretreatment time is included in the total run of the dissolution test method. For example, if the sample time of the test method is 45 min and the pretreatment time was 15 minutes, the sample time will be 30 minutes after the addition of the surfactant to the medium, thus maintaining the sample time at 45 minutes as stated in the method.

A few of the commonly used surfactants used in dissolution testing are sodium dodecyl sulfate, Triton-X-100 (polyethylene glycol tert-octylphenyl ether, Sigma Aldrich), and lauryldimethylamine N-oxide.

Physical explanation of aberrant results

When low release values are found in Tier 1 testing due to cross-linking, the addition of enzymes will almost always prove successful in obtaining results within specification. Only with the most severe degree of cross-linking will out-of-specification (OOS) results be obtained in Tier 2 testing. It should be noted, as shown in **Table IV**, the *USP* criteria for two-tiered testing does not have upper limits. OOS results are for low release values below the specification limits. Any result obtained above 125%, for example, would be considered aberrant and may require an investigation.

Table V shows actual data obtained in quantitative rupture testing of a pharmaceutical product. The specification for this product is 75% (Q) at 45 minutes. It is seen that the criterion for Tier 1 Stage 3 was not met at Tier 1 Stage 1 because there are release values below 50% (Q-25%). There was no need to continue with stages 2 and 3 of Tier 1 because no additional testing could result in passing Stage 3 criteria. Tier 2 testing resulted in acceptable data at Stage 1.

In **Table VI** are data from another softgel product on stability after nine months at 25 °C/60% relative humidity (RH)

Table V. Tier 1 failure at Tier 1 Stage 1 with specification met at Tier 2 Stage 1.

	% LC Released	
Vessel #	Tier 1 Stage 1	Tier 2 Stage 1
1	**11**	101
2	**16**	101
3	66	103
4	71	113
5	**23**	104
6	108	105
Mean	49	104
%RSD	78.7	4.1
Min	11	101
Max	108	113

Table VI. Aberrant high values at Tier 2 testing.

	% Released		
	Tier 1 Stage 1	Tier 2 Stage 1	
Vessel #	45 min	45 min.	75 min.
1	80	121	98
2	66	**165**	115
3	34	**152**	108
4	35	120	99
5	18	133	101
6	36	122	100
Average	45	136	104
RSD	52	14	6.4
Max	80	165	115
Min	18	120	98

conditions. The release specification of the product is 80% (Q) at 45 minutes. The product fails dissolution in Tier 1 testing at Stage 1. Although the product passes the criteria for Tier 2 testing at Stage 1 as shown in **Table VI**, there are some high aberrant values at the sampling time point of the test method (i.e., 45 minutes). An additional sample was taken at 75 minutes, and the results were acceptable values for the product. The capsules are not super-potent and non-uniform as might be suggested by the 45-minute sample. The high results obtained at 45 minutes are explained by the difference between the time the oil-containing drug is released from the ruptured capsule and the time the oil droplets are taken up by the surfactant micelles.

Surfactants form micelles when added to the dissolution media above their critical micelle concentration. Micelles are spherical in shape and have diameters on the order of 10–50 nanometers. During rupture testing, an oil droplet emerging from the capsule shell will have a diameter on the order of 0.25–0.5 millimetres. A micelle with a diameter of 30 nm has a volume of 1.4 x 10-20 mm^3. An oil droplet with a diameter of 0.25 mm has a volume of 8.2 x 10-3 mm^3. The volume of the oil droplet is on the order of a quadrillion times that of the micelle volume. However, there are many micelles capable of taking up all the oil released from the capsule. The oil in a typical softgel fill will be from 250–350 mg. The amount of surfactant added to the dissolution medium is on the order of 10s of grams. **Table VII** shows examples of dissolution methods from the FDA database for softgel capsules.

When an oil droplet is released from the capsule, there is a finite amount of time it takes for the tiny micelles to take up the large oil droplets. If the time when an oil droplet is released from the capsule shell is too close to the sampling time, the drug distribution in the dissolution medium may not be homogeneous because the oil droplets have not been fully taken up and absorbed by the micelles. In this case, sample taken may include an oil droplet not yet taken up by the micelles, resulting in high aberrant results.

As an example, say the sample volume is 1 mL and the dissolution medium is 1000 mL. If a drug product contains 2 mg of API in 250 mg of oil fill, the even distribution of drug at full release in the dissolution medium would be 2 µg/mL. But, if an oil droplet containing the drug has not been fully taken up by the micelles because the time between capsule rupture and sample time is insufficient, high release results will be obtained if any small oil droplet is included in the sample volume. Two mg of API in a 250 mg of capsule fill is 8 mg/mL. This means a droplet size of only 0.25 µL taken up during sampling will contain 2 µg of drug result in a drug release of approximately 200% label claim. A 0.25% droplet in a 1 mL sample size would be hard to visually detect in the sample.

Table VII. Examples of dissolution methods for softgel capsules (7). *USP* is *United States Pharmacopeia*. Source: FDA.

Drug name	Dosage form	*USP* appartus	Speed (RPMs)	Medium
Paricalcitol	Capsule	I (Basket)	100	4 mg/mL (0.4%) lauryldimethylamine N-oxide (LDAO)
Ergocalciferol	Capsule	II (Paddle)	100	0.5 N sodium hydroxide (NaOH) with 10% Triton-X-100 (polyethylene glycol tertoctylphenyl ether, Sigma Aldrich)
Dutasteride	Capsule (Soft-Gelatin)	II (Paddle)	50	Tier I: 0.1 N hydrochloric acid (HCl) with 2% (w/v) sodium dodecyl sulfate (SDS) (900 mL) Tier II: 0.1 N HCl with pepsin (as per USP) (450 mL) for the first 25 minutes, followed by addition of 0.1 N HCl with SDS (4% w/v) (450 mL) for the remainder of the dissolution test.

Conclusion

Dissolution testing of softgel capsules is better described as quantitative rupture testing because the drug is already dissolved in the oil fill of the drug product. The critical dissolution that occurs in quantitative rupture testing of softgel products is dissolution of the capsule gelatin shell. When cross-linking of the gelatin shell occurs, release of the drug from the capsule will be retarded. The impact of cross-linking on bioavailability is not significant if it can be shown that the drug release is overcome by addition of enzymes to the dissolution medium. If during Tier 2 release testing, drug release is below specifications, cross-linking is most likely severe enough to affect bioavailability. If, on the other hand, aberrant high dissolution results are obtained, the high results are indicative of release of drug from the capsule at a rupture time that is close to the sample time. Subsequent sampling beyond the sample time of the test method will result in assay values more in line with the product label claim.

It is important to understand results obtained from quantitative rupture testing of softgel capsules. Aberrant high values during quantitative rupture testing should not be interpreted as super-potent drug product or non-uniformity of dosage units. However, aberrant high results may still require careful consideration and possibly a laboratory investigation. Low results obtained during Tier 2 testing with enzymes may signal a possible problem with *in vivo* bioavailability. Experience with quantitative rupture testing is especially important during stability testing in understanding the performance of the drug product.

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Development — *Contin. from page 18*

Regulatory advantages

PTE: Do lipid-based systems provide advantages in terms of time to achieve regulatory approval?

Fulper (Catalent): Lipids mimic food and participate in the body's natural digestive process. In this sense, when you use lipids you are trying to create a food effect (with less calories). In the end, other approaches may work as well or even better, but in my opinion, working within the body's natural absorption process is a good starting point. Once you have a better picture of the pharmacodynamics involved, you can then decide if another approach might make sense.

From a regulatory perspective, if you plan to produce a generic version of a branded lipid formulation, there may be less concern if you maintain the same or similar formulation approach. Also, [it is helpful that] there is a level of intrinsic abuse deterrent for lipid-based formulations compared to some other delivery platforms.

McNaughton (Lonza): From a regulatory standpoint, most bioavailability enhancement technologies are now considered to be well understood and accepted. Generally, any technology that is used to improve bioavailability should aim to minimize the overall quantity of drug substance required and minimize the variability in the patient population. Where the cannabinoid is poorly soluble in water and highly lipophilic, then lipid systems offer the best opportunity to achieve this, making it the most likely approach to be readily approved by the regulators.

Future trends

PTE: What future trends do you predict for solubility and bioavailability enhancement options for cannabinoids?

McNaughton (Lonza): More formulations may appear that have been refined to achieve the best bioavailability for each cannabinoid. Beyond this, more synthetic cannabinoid analogues may be produced, either as prodrugs or to modify other properties of the molecule. It is possible that many of these analogues will be derived to promote properties that can be used to enhance bioavailability, which for many is likely to be combined with lipid technologies to maximize efficacy with minimal drug substance. Others may be derived to improve selectivity and efficacy in specific areas, and these may still require significant formulation development to ensure bioavailability.

Fulper (Catalent): As the pharmacology of cannabinoids and the endocannabinoid system become better understood, I would expect synthetic cannabinoid derivatives to begin to appear. As part of that process, I think you will see a focus on molecular changes to improve stability and reduce metabolism issues, which are probably the two main challenges with the existing analogues.

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Designing Commercial-Scale Oligonucleotide Synthesis

Current large-scale practices can be considered as a basis for improving multi-product manufacturing platform strategies.

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The strategy of targeting RNA to control processes affecting disease pathology has seen a flurry of clinical success. Regulatory approvals of Spinraza (nusinersen, Biogen), Exondys 51 (eteplirsen, Sarepta Therapeutics), Tegsedi (inotersen, Akcea Therapeutics), and Onpattro (patisiran, Alnylam Pharmaceutials) in the past few years highlight the relevance of the technology as a new platform for treating disease.

The manufacturing processes being used to produce several of these molecules are built on the solid-phase phosphoramidite approach developed by Beaucage and Caruthers more than 35 years ago. Since then, there has been significant improvement and optimization of the raw and starting materials, reaction parameters, purification, and isolation processes used for oligonucleotides and oligonucleotide mimics. The application of these improvements in large-scale (i.e., multi-kilogram batch) scenarios is becoming more common as more is learned about translating bench-scale methods to a commercial-scale manufacturing setting.

The oligonucleotide field, which is still in its commercial infancy, does not have decades of commercial-scale manufacturing experience on which to draw, but it is beginning to transition to this level of large-scale process experience, as evidenced by drug approvals and new plant capacity. The objective of this article is to provide a framework for developing and improving manufacturing strategies used for oligonucleotides, beginning with an overview of how oligonucleotides are currently synthesized at large-scale.

Some companies internalize their manufacturing capabilities, while others turn to contract manufacturing organizations (CMOs) for process design, scale-up, and implementation. Regardless, the manufacturing facility is most likely to be a multi-product one. There is not yet an oligonucleotide drug requiring significant enough quantities (i.e., metric tons) to warrant a compound-dedicated plant, but most of the companies involved in the manufacturing processes are banking on the success of the oligonucleotide platform to warrant further capital investment. Therefore, the recommendations in this article are directed toward the multiple-product facility where platform processes are paramount for rapid scale-up and minimal compound-specific process research.

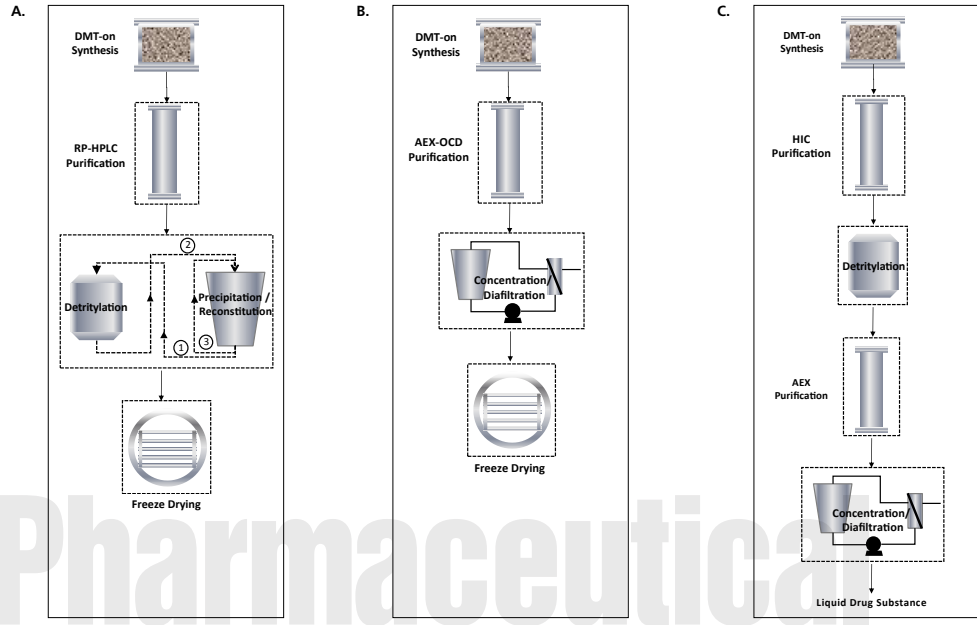
Oligonucleotide manufacturing overview

In its simplest description, oligonucleotide manufacturing consists of only a few, basic unit operations: solid-phase synthesis, purification, and isolation. The earliest syntheses of oligonucleotide drug substance were performed via solid-phase couplings on controlled pore glass (CPG) in sparged bed reactors. While sparged bed reactors are still common and practically useful in high-throughput and small-scale applications, the extensive amount of solvent wash and reagent excess required to accommodate that mixed-bed reactor design are not desirable in larger-scale operations where economic and logistical considerations are important.

Packed bed reactors (PBRs) and higher yielding polymeric, swellable resins (1,2) can accommodate the significant mass addition of a typical oligonucleotide (7 kDa). PBRs are used commonly outside the pharmaceutical industry in chemical applications in which a bed of solid catalyst is interacting with a liquid or gas reactant. Although the phases for oligonucleotide synthesis differ from traditional PBRs (i.e., liquid-solid vs. gas-solid), the same basic design principles apply. Characteristics of PBRs such as residence time distributions (3) and other fluid mechanical characterizations have been described recently (4,5). These characterizations represent an opportunity for incremental improvements in large-scale oligonucleotide synthesis.

Following solid-phase oligonucleotide synthesis, crude material is purified and isolated.

Figure 1: Large-scale oligonucleotide processes in-use. a. Process 1 is used for all Ionis compounds (non-GalNAc) and has been validated for three commercially approved products. b. Process 2 is used at a contract manufacturing organization and has been commercially validated for one Ionis compound. c. Process 3 is Biogen's process presented at TIDES 2018.



Among the purification options being employed at large-scale are reversed-phase high performance liquid chromatography (RP-HPLC), ion exchange chromatography (IEX), and hydrophobic interaction chromatography (HIC). RP-HPLC and IEX are being used for several of the recently approved oligonucleotide therapeutics and thus have a proven record in commercial manufacturing. HIC is a recently described (6) and interesting addition to the toolbox because it operates on principles analogous to RP-HPLC but without the use of organic solvent. Despite the different principles of separation, the three techniques have the same gross effect on the crude mixture. That is, they remove shortmers (low molecular weight oligomers) that arise from incomplete coupling during synthesis or side reactions that lead to short cleavage products and longmers (high molecular weight oligomers) that arise from starting material-related impurities. Although there may be subtle impacts made to impurities closely related to the product (e.g., missing one nucleotide), the bulk of the purity improvement is from a reduction of impurities significantly shorter or longer than the parent molecule. Therefore, the rationale for choosing a purification technique will rest, in large part, on the previous experience, existing equipment, or a combination thereof at the CMO or company executing the large-scale work. For example, retrofitting an existing facility or repurposing equipment not rated for flammable solvent will almost certainly push the solvent-heavy RP-HPLC process to the bottom of the list and move the typically aqueous buffer systems of IEX and HIC to the top of the list.

Regardless of the purification technique employed, oligonucleotide must be isolated from the purification buffer and, in some instances, converted to the appropriate salt form. In nearly all applications, the final isolation step is designed to result in a solid form for logistical, stability, and operational convenience. Ethanol precipitation and ultrafiltration (i.e., diafiltration + concentration) are the intermediate isolation options, and freeze drying is almost always used for the final isolation. In comparison to ultrafiltration, which can accommodate nearly any purification buffer system, ethanol precipitation has limitations on the feedstock solution matrix and oligonucleotide concentration. Therefore, the intermediate isolation operation will be inextricably linked to the method (IEX, RP-HPLC) and mode (step, linear) of chromatography.

Three large-scale processes in-use today are presented in **Figure 1**. It should be noted that all three leave the final 4,4′-dimethoxytrityl (DMT) protecting group intact at the end of synthesis, which necessitates that it be removed downstream. In the case of Process 1 and Process 3, removal is accomplished via a solution-phase detritylation reaction. In contrast, Process 2 performs the detritylation reaction on the purification column, thus combining the purification and deprotection steps into a single unit operation. The overwhelming amount of large-scale data at the authors' disposal are from Process 1. Recent

ALL FIGURES COURTESY OF THE AUTHORS.

Figure 2: Large-scale synthesis yields. The symbols represent the different chemistries where e = 2'-O-methoxyethyl (MOE), d = deoxy, k = constrained ethyl (cEt).

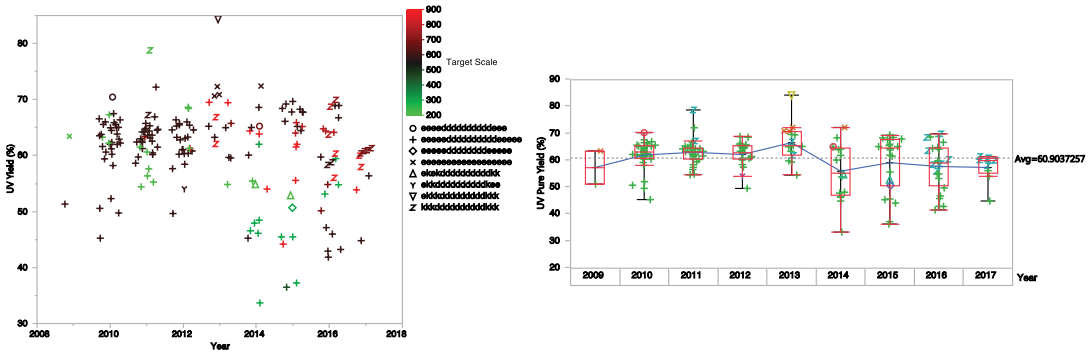
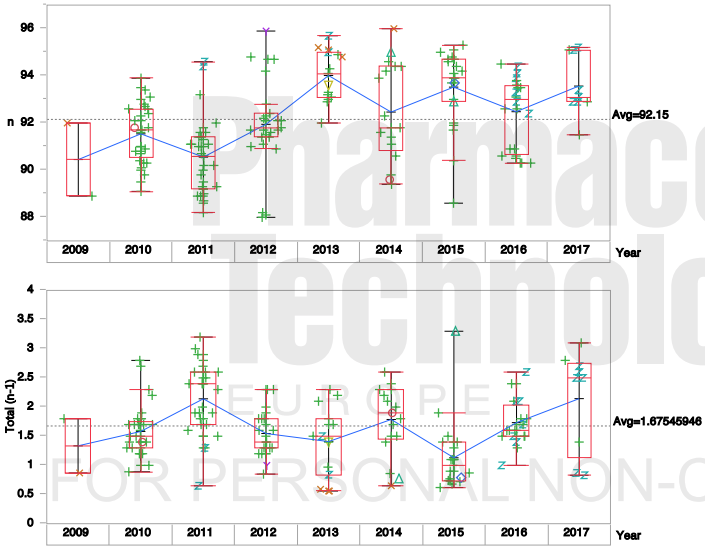


Figure 3: Crude mass spectrometry purity and total (n-1) levels.



large-scale experiences with Process 2 are also presented. While Process 3 is only recently described, its novel use of HIC and delivery of liquid drug substance make it worth noting, although it has not been validated for a commercial process.

Each of the chemistries used in RNA-based therapeutics (e.g., deoxy, 2'-*O*-methoxyethyl modification [MOE], constrained ethyl [cEt], RNA, morpholino) present their own challenges, but the basic unit operations used among them are similar. Focusing on oligonucleotides such as those produced from processes presented in **Figure 1** narrows the scope of the data but not the basic design principles. Assuming a multi-product oligonucleotide facility producing hundreds of kilograms of drug substance a year is an appropriate, realistic, and useful base case for process design. This article focuses on the solid-phase synthesis step of the manufacturing process, which is the same in all three processes.

Chemical and analytical considerations

Solid-phase oligonucleotide synthesis consists of four basic steps: 1) detritylation to remove the 4,4'-dimethoxytrityl (DMT) protecting group, 2) coupling to attach the activated phosphoramidite, 3) sulfurization or oxidation to convert the internucleotide linkage to either a phosphorothioate or phosphate diester, and 4) capping to prevent any unreacted sites from elongating further. At the end of synthesis, cyanoethyl protecting groups are removed from the phosphorus with an amine solution in acetonitrile (MeCN). Support-bound oligonucleotide is then cleaved, and nucleobases are deprotected in aqueous ammonium hydroxide at elevated temperature. Aside from a single commercial product, Ionis exclusively uses the same ion-pair HPLC with ultraviolet (UV) detection coupled to mass spectrometry (IP-HPLC-UV–MS) analytical method for analyzing oligonucleotide. The method utilizes the UV dimension of the analysis for quantifying impurities significantly shorter and longer than the parent molecule and MS for impurities of similar length.

Large-scale data

In large-scale synthesis operations at Ionis' oligonucleotide plant, a variety of process parameters and outputs are tracked as part of production, but the fundamental questions of how much and how pure are most useful for those interested in rugged process design and logistical planning. On average, the UV-pure yield from synthesis is 61%. UV-pure yield refers to the full-length and nearly full-length products (e.g., n-1, where n is the parent molecule). This measure of yield is useful because Ionis' method

Figure 4: Synthesis data for a single Ionis compound.

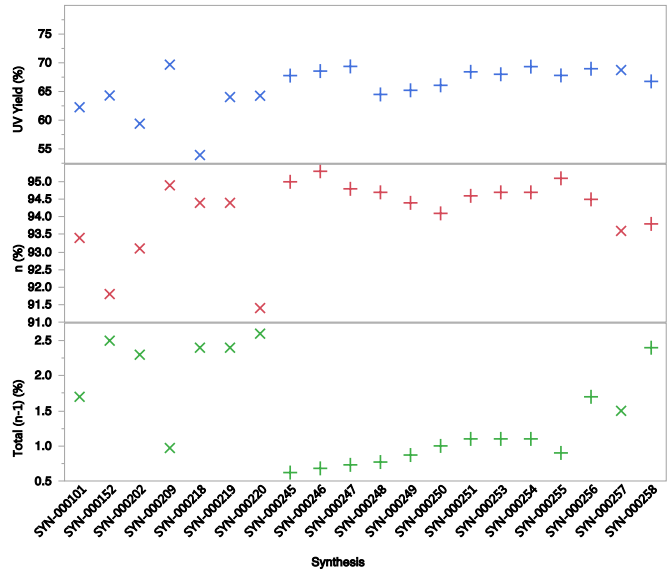
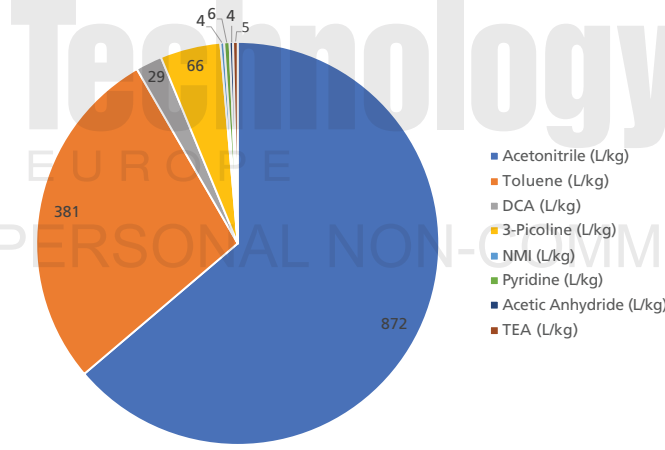


Figure 5: Solvent consumption for 4×600 mmol syntheses of 5-10-5 2'-O-methoxyethyl (MOE) deoxy gapmer (Note: drug substance batch size was 11.488 kg)



of impurity characterization via MS is not ubiquitous, but chromatographic characterization is. Most of the data used to arrive at the 61% average come from 5-10-5 MOE (the 2'-*O*-methoxyethyl modification) deoxy gapmer compounds, which translates to an average coupling efficiency of 97.56% (0.975620=61%). This is an impressive value considering the near stoichiometric amount of phosphoramidite used in the coupling reaction (1.4 eq.).

The average 5-10-5 compound has a sodium salt molecular weight of 7.7 g/mmol, thus a 100% downstream recovery would translate to an isolated yield of 4.7 g/mmol of synthesis. This value is useful to have in hand for planning purposes. For example, a metric ton of a 5-10-5 would require a minimum of 213 moles of synthesis, but accounting for downstream loss would require more likely 250 moles of synthesis. In approximately the past decade's worth of data, the yields have been relatively consistent across varying compound motifs and synthesis scales as illustrated in **Figure 2**.

Using Process 1 presented in **Figure 1**, the majority of the early and late eluting impurities are removed in downstream processing, but nearly all of the co-eluting impurities quantified by MS are retained. Thus, the MS impurity profile of the crude is a near perfect indicator of the drug substance MS impurity profile. The other processes presented in **Figure 1** can afford some modest reduction in co-eluting impurities, but the results at the crude stage will still largely determine the overall MS purity. On average, crudes produced at Ionis have an MS purity of 92%. There has been an upward trend in MS purity over the years (**top panel, Figure 3**) with greater understanding of process-related impurities. The single largest class of impurity contributing to the impurity profile is (n-1), which has also had significant variability over the years **(bottom panel, Figure 3**). This variability will translate directly into the drug substance in Process 1 because these impurities are carried through downstream processing. In theory, Process 2 could have the potential to remove these impurities due to their lower overall charge (i.e., number of charges for interaction with the stationary phase), but in our limited experience we have not observed this to be reliably or practically true. Although the author does not have large-scale data for Process 3, one of the recent patent claims is that by careful column loading, HIC can reduce selected (n-1) impurities (7).

In general, Ionis' practice is to apply platform process experience to new compounds and begin to develop more compound-specific synthesis methods while moving through development into process performance qualification (PPQ). In **Figure 4** are data for a single compound where the × markers note syntheses where the synthesis process was being varied and the + markers note a fixed, compound specific method for synthesis. The fixed method was developed based on compound-specific synthesis studies and, as is apparent in the figure, led to less variation in yield and purity.

Table I. Waste and solvent consumption per kg of drug substance at Ionis plant using Process #1 (see Figure 1). MeCN is acetonitrile.

Year	Waste (L/kg)	MeCN (L/kg)	Toluene (L/kg)
2009	3197	1393	540
2010	4276	1393	540
2011	4108	1393	540
2012	3921	1393	540
2013	4461	1334	479
2014	6157	1538	460
2015	4985	1074	441
2016	4529	992	452
2017	5170	1603	605
2018	3358	1087	540
Avg.	4416	1320	514

The data set is considerably smaller, but large-scale results for Process 2 (**Figure 1**) are similar to Process 1. Unfortunately, the crude analytical method used for Process 2 differs from Process 1, thus making direct comparison difficult. Grossly, the crude purity was slightly higher and the isolated yield slightly lower.

Quantity and quality of material are of paramount importance, but waste and solvent consumption are also worth considering as the demand of oligonucleotide increases. As an example of synthesis solvent consumption, consider four of the syntheses (SYN-000245, 246, 247, 248) that were combined to form a single lot of drug substance. The as-is yield for this lot was 11.488 kg or 4.79 g/mmol. The precise consumption of solvent was totaled and normalized by the lot size in **Figure 5**. As might be expected, the two largest sources of solvent consumption were toluene and acetonitrile. The consumption, however, only considers solvent consumed during the synthesis and does not include solvent used for cleaning, equipment maintenance, reagent heels, etc. Projecting true consumption requires knowledge of the precise batch sizes, tank volumes, and cleaning procedures. For example, four 10-kg campaigns would likely require more cleaning and reagent preparations than a single 40-kg campaign. As a rough estimation of true solvent consumption across the plant (Process 1), volumes consumed and waste generated are tabulated for Ionis' plant in **Table I**. So, although the campaign described in **Figure 5** consumed 872 L/kg of acetonitrile during synthesis, the yearly consumption of acetonitrile suggests that 1320 L/kg is a more appropriate value for estimating total plant usage.

Process development

The platform nature of oligonucleotide manufacturing allows for rapid scale-up with minimal process development. The ruggedness of the platform processes being employed is highlighted by consistently high yields and short production times across a range of compounds and chemistries. The average production time from synthesis column packing to unloading the freeze dryer is 67 days, accomplished with a five-day work week and a single shift of engineers. Production times as short as 19 days have been achieved by eliminating intermediate testing, which highlights the efficiency of actual unit operations as opposed to the testing and quality aspects of production.

Prior knowledge and experience have enabled initiation of large-scale, GMP production immediately with little to no laboratory-scale process development. As a compound progresses through clinical development toward commercial production, compound-specific experiments are performed to establish optimal process control set points and proven acceptable ranges (PARs) for PPQ. In establishing these ranges, tight PARs based on historical equipment performance were often used, rather than PARs on what the chemistry could accommodate. Most of the deviations and process failures to date have been associated with equipment failure as opposed to a failure of the chemistry or process parameters. Thus, in choosing a process, simplification by limiting the number of manufacturing steps would be a route to minimizing process failure by reducing the number of potential mechanical failures and equipment errors.

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How Advanced Mass Spectrometry Technologies and Workflows Are Delivering Comprehensive Protein Characterization

Complex protein structures pose analytical challenges that can be addressed by advanced mass spectrometry technologies and workflows, which can be used to comprehensively characterize them.

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Advances in protein engineering have empowered pharmaceutical developers to optimize and exploit the therapeutic potential of proteins much more quickly and cost effectively than usual, while maintaining or even enhancing their safety profile (1). Innovative recombinant protein therapeutics, such as monoclonal antibodies (mAbs), fusion proteins, and antibody-drug conjugates, are now being used to treat an expanding range of conditions, including cancers, inflammatory, autoimmune, and genetic diseases. Structurally larger in size than small-molecule drugs and functionally dependent on post-translational modifications (PTMs), these classes of therapeutics bring added complexity to the protein characterization process.

With greater protein complexity comes the need for robust analytical methods. Current analytical technologies, workflows, and data processing methods need to accommodate the requirements for biotherapeutic protein characterization (i.e., confirming identity and detecting PTM status for each residue, and measuring abundance of both major and minor intact protein isoforms to ensure the quality and consistency of these products). Such a thorough characterization effort would not only help protect patients for whom the drug will be developed, but also reduce the time required to bring these protein-based therapeutics to market. A robust, reproducible characterization protocol also functions as a quality control step to measure batch-to-batch variability of the drugs. As with all high-throughput fields, giving researchers the ability to automate time-consuming steps will make the characterization process even more productive.

This article considers the challenges associated with determining the structure of complex protein biotherapeutics, and how researchers can employ the latest mass spectrometry (MS) technologies and workflows to confidently characterize these increasingly important drugs in an efficient and comprehensive manner.

The challenge of complex protein therapeutics

Recombinant protein therapeutics are intricate molecules, typically orders of magnitude larger in size than traditional small-molecule drugs. The production of these biotherapeutics relies on living cells or organisms that are extremely sensitive to a range of factors, such as species origin and culture conditions (1). As therapeutic efficacy often requires preservation of precise secondary or tertiary molecular structures, comprehensive characterization is required during development and manufacture. Gaining such structural insights can be difficult when synthesis is complicated—often requiring over 5000 critical process steps to generate a recombinant biotherapeutic (2).

Given the need to assure the safety and quality of these therapeutic products, there is a growing requirement for reliable analytical methods capable of quickly characterizing the structure of these molecules. There is also a need for these methods to reduce the number of test strategies required and the potential for operator error. Moreover, with the growing number of hybrid therapeutic products under development, such as antibodies fused to highly glycosylated or otherwise analytically challenging molecules, the technologies and workflows used by researchers to confirm the structure of these complex molecules must keep pace with the evolving biopharmaceutical landscape.

Comprehensive protein characterization typically involves a combination of intact mass analysis and peptide mapping to confirm both the total mass of the species and elucidate the substructure in fine detail. Ongoing improvements to the capabilities of high-resolution accurate mass (HRAM) analyzers and complementary dissociation techniques, as well as increasingly reliable and efficient protein

digestion solutions, have enabled highly effective peptide mapping workflows that can probe protein structure at the individual amino acid level. Additionally, recent advances in MS technology are now enabling powerful non-denaturing liquid chromatography–mass spectrometry (LC–MS) methods for intact mass analysis, reducing the reliance on orthogonal, non-LC–MS-based approaches. These so-called "native" LC–MS strategies are enabling researchers to support the characterization of microheterogeneous isoform mixtures of covalently assembled molecules. The combined analysis directly correlates identified features to each proteoform, removing the need for manual inferences.

Traditionally, denatured, intact mass analysis generates convoluted MS spectra containing a mix of intact mass isoforms and wide charge state envelopes with much greater charge state overlap, which result in the production of complex mass spectra. Although spectral deconvolution algorithms can be used to interpret this complexity, these computational workarounds have their limitations. Performing MS in non-denaturing conditions, a characteristic feature of native MS, can help mitigate these issues. By spraying the intact protein sample in physiologically approximate buffers consisting of volatile salts, native MS enables the protein to retain its native structural characteristics and, in effect, shield internal basic residues from becoming protonated in a typical positive mode MS analysis. Folded protein isoforms have decreased charge states and increased spectral separation between each of the isoforms in a mixture, resulting in relatively simplified mass spectra. With native MS, it is often possible to achieve the resolution of intact protein isoforms, such as mAb glycoforms, without the need for sample pre-treatment.

Coupling native MS with separation techniques that are compatible with "native" mobile phases, such as size-exclusion chromatography (SEC), provides a convenient and automated analytical workflow that can effectively resolve the complex protein architecture. As a result, native SEC–MS intact mass analysis can deliver cleaner spectra and provides reliable characterization data in a high-throughput fashion.

Advanced MS tools for intact mass protein characterization

To characterize complex proteins in their intact state, in addition to optimizing the native MS methods, significant efforts are made towards charge reduction techniques with the goal of obtaining improved resolution. In recent years, advances in SEC–MS intact mass analysis have been driven by the advent of solution-phase charge reduction additives, such as triethylammonium acetate (TEAA). Due to the high pKa values associated with these additives, these reagents enable protons to be efficiently abstracted from the protein, reducing the overall charge (*z*) on the analyte. The lower value of *z* imparted by the additives shifts the mass-to-charge ratio (*m/z*) distribution, resulting in higher *m/z* ratios and better spectral separation. Higher concentrations of additive can facilitate enhanced charge reduction and improve the spectral separation of intact protein isoforms. However, a frequent challenge encountered when working with solution phase additives is the need to regularly clean heated capillaries because the build-up of material can quickly have a detrimental impact on analytical performance.

Proton transfer charge reduction (PTCR), an alternative technology using gas phase ion source reagents, can help overcome the challenges posed by solution-phase additives. The ion–ion proton transfer reactions serve as an effective method for gas-phase charge reduction, yielding cleaner spectra while minimizing maintenance requirements. Recently, PTCR has become commercially available, providing researchers with wider access to this technology in a vendor-supported format. For example, an advanced ion source and modified dual-pressure linear ion trap (e.g., such as that only available in the latest Thermo Scientific Orbitrap Tribrid mass spectrometer) provides significantly increased ion–ion reaction efficiency, which provides support for enhanced PTCR capabilities. Through the application of other proprietary innovative ion management technologies, including hardware that more precisely manages electrical fields and reduces noise, these designs can maximize ion transmission from injection to detection, delivering robust qualitative and quantitative performance. Such improvements can eliminate electrospray ionization (ESI) source contamination and simplify the MSn spectra of intact proteins and complexes, ultimately increasing confidence with intact and top-down protein sequencing.

Advanced workflows

MS instrumentation. The use of PTCR as a powerful strategy for intact mass analysis has been further facilitated by the extended high-mass-range functionality offered by some modern instruments. The latest Orbitrap Tribrid system, for example, is now capable of performing high mass measurement and isolation up to 8000 *m/z*, enabling the higher-order analysis of large-protein complexes and their components. Moreover, this next-generation system is capable of supporting both peptide mapping and intact mass workflows in a single platform. Fragmentation techniques with higher energy collisional dissociation (HCD) and electron transfer dissociation (ETD) facilitate more detailed peptide mapping, while automated PTCR-enabled charge reduction applied to native MS yields improved resolution for intact mass analysis. Using ion trap isolation to further isolate smaller *m/z* windows helps significantly improve the signal-to-noise ratio, allowing for more control in the experimental workflow to characterize complex biotherapeutics. Native MS coupled with sequential enzymatic dissection of individual subunits in highly glycosylated proteins, such as etaner-

cept, can offer additional information on glycan heterogeneity compared to bottom-up peptide mapping, thereby acting as a fingerprinting tool to asses batch-to-batch variability of drugs (3).

The features of this advanced MS instrument help scientists obtain the high-quality protein characterization data necessary to drive the right decisions along with the ability to expand their experimental capabilities in the future.

Data acquisition. Further advances in MS technologies and workflows are helping researchers confidently characterize protein therapeutics more quickly. The latest intelligence-driven data acquisition strategies, such as charge state directed dissociation, are delivering improved analytical specificity and making data collection significantly faster. By enabling spectra to be processed using automated precursor determination, precursor charge state analysis can be performed in real-time, enabling the intelligent selection of the dissociation techniques to be employed as well as the optimal parameter settings for high-quality MS^n acquisition. For example, higher charge state precursors may fragment significantly better using a combination of ETD and higher-energy collisional dissociation (EThcD), requiring both the duration of ion–ion reactions and relevant collisional activation energy to be imparted. Intelligent MS methods have been further extended utilizing data-independent acquisition PTCR to be implemented following ETD spectral acquisition. These workflows can boost efficiency and accelerate the generation of high-quality results through more complete MS^n spectral acquisition and confident, automated data processing.

Data processing. Newly developed data processing tools, used in conjunction with state-of-the art MS systems, enable accurate interpretation of results. For example, the Sliding Window algorithm (Thermo Scientific BioPharma Finder software) used for deconvolution of intact proteins "slides" along a chromatogram, acting as an overlay to generate time-integrated results (4). Traditional intact protein deconvolution involved averaging all the spectra corresponding to an arbitrarily selected chromatographic time. However, with numerous LC peaks eluting over time, averaging all the spectra in a selected time doesn't accurately represent the protein isoforms present in separation. By interrogating smaller windows within a larger chromatographic range, modern software algorithms perform deconvolution multiple times in succession, resulting in a more accurate understanding of how the spectra behave and change over time. As the set window width moves along the LC time axis, different isoforms in the chromatographic separations can be detected multiple times. Redundant detections improve the confidence of reported isoforms, in terms of both mass and abundance. Using the minimal percentage offset enables the greatest number of redundant detections, thus allowing the detection of even minor components that may elute for shorter times. Applying these approaches to characterize biotherapeutic proteins allows researchers to analyze mass spectra of different protein isoforms with varied elution profiles. Performing deconvolution using the Sliding Window algorithm can, for example, provide more accurate drug-to-antibody (DAR) ratios, taking even the lower abundance species into account when analyzing antibody-drug conjugates.

The future of protein characterization

A few years ago, analyzing an intact complex protein sample would have yielded a mass spectrum that would pose significant deconvolution challenges, making it difficult to be analyzed or interpreted. The latest MS systems, with the capacity for native analysis and charge reduction with PTCR, have opened up the possibility of comprehensive and automatable characterization of highly complex proteins in a single platform. However, with the ability to isolate every portion of the spectrum and closely examine the charge state distributions of the isoforms present, researchers must take care not to introduce implicit bias to their analysis. The application of the latest technologies can eliminate the risk of a skewed isoform distribution, especially when characterizing therapeutic proteins, relieving the possibility of introducing any bias.

The nature of protein therapeutics will continue to evolve as the field of protein engineering accelerates. Simultaneous advances in MS technology will ensure researchers have access to analytical tools that can keep up with the increasing complexity of modern biotherapeutic products.

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Best Practices in Using Isolator Technology

Consider equipment design, transfer systems, and maintenance when operating isolators for sterile manufacturing of pharmaceutical products.

Jennifer Markarian

In sterile manufacturing and aseptic fill/finish of pharmaceutical products, isolator technology offers the ability to achieve high sterility levels. *Pharmaceutical Technology Europe* spoke with two experts to learn about best practices in specifying and operating isolators. Richard Denk is senior consultant for Aseptic Processing & Containment at SKAN AG, which designs and manufactures isolators, isolator process solutions, and cleanroom equipment. Denk founded the Parenteral Drug Association (PDA) Isolator Expert Group, which has published guidelines for isolator design and cleaning. Denk was also responsible for writing about transfer systems and isolator design for PDA's technical report on isolators, which will be published in 2020, and is chair of the International Society for Pharmaceutical Engineering Germany/Austria/Switzerland (ISPE D/A/CH) Affiliate's Containment Expert Group, which published the ISPE *Containment Manual*. Steve Nole is vice-president of operations at Grand River Aseptic Manufacturing (GRAM), a contract development and manufacturing organization (CDMO) for parenteral pharmaceuticals. GRAM is building a new aseptic processing facility, near its existing facility in Grand Rapids, Michigan, United States that uses isolator technology from SKAN. The company is currently in the process of equipment qualification, with good manufacturing practice (GMP) production planned for start-up in September 2020.

Isolators vs. RABS

PTE: What do you see as the advantages of isolator technology for aseptic manufacturing? When are isolators a good choice compared to restricted access barrier systems (RABS)?

Nole (GRAM): The main advantage for isolator-based technology is that it removes the most significant source of contamination from the aseptic environment by eliminating direct interventions by gowned employees. Typically, with an isolator system, a higher sterility assurance level (SAL) is achieved. Also, isolators can be placed in a Grade C surround, which requires less cleanroom real estate, whereas RABs require a higher level of air classification and additional airlocks, more stringent gowning requirements, and support to operate and maintain a Grade A/B RABS system.

Both isolators and well-designed RABS systems can achieve very high SAL. Isolators may be a better choice for a new greenfield building because you can achieve a higher SAL, operate in a Grade C space, and recognize lower operating expenses due to a smaller cleanroom and less gowning requirements; however, isolators require a higher initial investment for equipment costs. RABS may be best suited for upgrading existing equipment already within a Grade A cleanroom. Lastly, a company may choose RABS/isolator lines based on the company's existing technology and training. For example, a company with existing RABS lines may stay with RABS for consistency and training of the workforce.

Denk (SKAN): Isolators most comply with the regulatory requirements of the pharmaceutical authorities, such as the US Food and Drug Administration (FDA) or the European Medicines Agency, with regard to sterile manufacture. If you look at the Draft Annex 1, which was published in December 2017, you will find the term 'isolator' 34 times throughout the document (1). Draft Annex 1 is a European document, but [global regulatory] members, such as FDA, WHO [World Health Organization], and PIC/S [Pharmaceutical Inspection Cooperation Scheme], are involved in this document, which also makes the document of international importance. The advantage of isolators is the barrier between the operator in production and the sterile product to be manufactured. The operator is seen as the highest contamination risk to the sterile pharmaceutical product. Compared to the RABS, which is decontaminated together with the room, the isolator has its integrated decontamination system, which offers significant advantages. As a result,

the aseptic area is also reduced inside the isolator chamber, decontamination can take place more quickly, and the decontamination cycle can be validated. This setup also has advantages for the operators, since they can work in a reduced GMP environment of ISO [International Organization for Standardization] class 7 or 8.

Specifying isolators

PTE: What are some of the keys in choosing/specifying isolators and surrounding equipment?

Nole (GRAM): Factors to consider include decontamination cycle time, changeover, residual vapourized hydrogen peroxide after decontamination, and where/how the vapourized hydrogen peroxide is introduced (below or above the HEPA [high efficiency particulate air filter]). Other significant items for GRAM were whether the isolator and equipment vendors worked together in the past, how many installations they have fully commissioned in the United States, and support and service capabilities. These factors become even more critical if the OEM [original equipment manufacturer] is responsible for routine maintenance and requalification activities.

Denk (SKAN): In the fill/finish area of sterile manufacturing, isolators are primarily selected and specified to ensure an aseptic environment in the filling process. In addition, if the pharmaceutical product is a highly active or highly hazardous substance, there are additional protective factors on the isolator to secure the operators during production. The topic of cleaning to avoid cross-contamination in a multi-purpose system is becoming increasingly important. The permitted daily exposure (PDE)/acceptable daily exposure (ADE) levels are getting lower; especially in the fill/finish area, any external contamination in the vial or syringe, for example, can quickly exceed the PDE/ADE. In order to prevent this, I founded a PDA expert group in 2015 that has now published two PDA publications on the subject of cleaning and avoiding cross-contamination. The first publication, *Isolator Surfaces and Contamination Risk to Personnel and Patient*, deals with the limit values for cleaning of non-direct product contact surfaces inside and outside of isolators with the associated equipment such as the filling line (2). In the second publication, *Preventing Cross Contamination During Lyophilization*, cleaning and its limit values for surfaces not in contact with the product within a lyophilizer were considered (3). In addition to these factors for the selection and specification of isolators, other factors such as the surrounding equipment also play an important role. How do the stoppers and caps get into the isolator? Or if ready-to-use (RTU) primary packaging, such as nested vials or syringes, are used, how are they introduced and the packaging removed?

Best practices

PTE: What are some ways to address challenges for moving material in and out of the system?

Denk (SKAN): Transfers in and out of an isolator are areas that should be examined closely in a risk assessment, because they have an impact on sterility and containment. The risk assessment should be performed in the beginning (during mock-up) and include GMP and EH&S [environment health and safety] requirements. Different decision criteria play an important role in the selection of the suitable transfer system. Is the intention for a large-scale production or smaller batch sizes, and mono-production or multipurpose production? Will reusable units such as a stopper processor or single-use packaged units be used? Is a high degree of flexibility required when selecting different format sizes, for different vials, for example? Once these decisions are made, there is the right transfer system for each unit. In 2020, PDA will publish a technical report for isolators. The report also describes, among other things, transfer systems and how they work safely in an aseptic environment.

Nole (GRAM): You need to have a robust system in place that provides flexibility. GRAM can use a combination of dedicated parts that are autoclaved and transferred into the isolator via rapid-transfer canisters, or preassembled and gamma-irradiated assemblies in bag-in-bag-out with rapid-transfer ports. This setup allows us to assemble and sterilize our own assemblies or source preassembled material from a third party.

PTE: What are some of the best practices for maintenance of isolator technology?

Denk (SKAN): The best practice for maintenance of isolators is predictive maintenance. The isolator is the aseptic barrier between the sterile product and the environment. The better this barrier works, the safer the sterile product. Some maintenance personnel are particularly distinguished in keeping the isolators in good shape for a very long time. I'm often surprised seeing isolators installed many years back that still look like new. We have a SKAN Academy where all of our service people are trained, as well as the operators of our customers if they have no experience with isolators or would like to enlarge their skills. Most of our customers have a service contract and are thus informed about their upcoming maintenance to be coordinated with their production cycle.

Nole (GRAM): One crucial best maintenance practice is a glove maintenance/management programme. Understand your glove change frequency and have six to 12 months of inventory on the shelf—gloves have long lead times.

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Managing Risks for Cell and Gene Therapy Logistics

Vein-to-vein programmes are focusing on data access and traceability.

Agnes Shanley

The past few years have seen dramatic growth in cell and gene therapy research and commercialization. Developers are adding new laboratory and manufacturing capacity to help keep up with explosive demand for new therapies. Over the past few months, Kite Pharma and Novartis have both built new facilities in the United States and the European Union, while Lonza, WuXi Biologics, and other contract development and manufacturing organizations (CDMOs) have been expanding manufacturing capacity.

Established ways of gathering, transferring, and storing data and materials for traditional biopharmaceutical and small molecules will not work in the evolving supply chain for autologous and allogeneic therapies. For one thing, manufacturing and transport involve many more diverse stakeholders and patient groups than traditional programmes, while, for autologous therapies, there is a need to closely coordinate raw material extraction and final product production, and the transport to and from collection centres and from manufacturing to clinic.

Recently, alliances have started up to streamline data management and access to improve the transmission of data from raw material collection points to manufacturing, and to patients at the point of use. In one such alliance, the CDMO Lonza, the information technology (IT) company Vineti, and logistics specialist Cryoport are working to optimize logistics for cell and gene therapies. Lonza's formal collaboration with Vineti had begun in July 2019 to integrate the CDMO's manufacturing execution system (MES) and electronic batch record (EBR) solution with Vineti's platform.

Built-for-purpose IT systems

Vineti, which focuses on supply chain management for cell and gene therapies, has been in business for approximately four years, and grew out of GE Ventures, says cofounder and CEO Amy DuRoss. Back around 2012, a leading pharmaceutical company with a chimeric antigen receptor T cell (CAR-T) product in Phase III had approached GE to help with unprecedented manufacturing and supply-chain issues. The product, which is now commercially available, was being produced almost entirely manually, says DuRoss, and presented the most complex supply chain and logistics workflow requirements ever seen in the history of biologics. "They were using brute force against the production and delivery of each therapeutic. Once they saw that they were likely to commercialize, they realized that there was no way to scale production and delivery on a manual basis," she says. For roughly two years, GE Ventures evaluated the challenges involved, and Vineti was set up in 2015 as an independent company to automate those specific supply chain and logistic workflow requirements. The company uses an enterprise platform developed inhouse, and an integration layer driven by application programming interfaces (APIs).

With CDMOs, the connection point depends on what the partner wishes to emphasize. With Lonza, for example, the emphasis is on the MES and EBR system. "Vineti is managing chain of identity and chain of custody externally, and providing data as a feed internally into Lonza's shop floor," DuRoss says. The partners plan to deepen the capacity for data sharing and integration to improve capacity optimization. "This will reduce the data integration burden on CDMO customers, since the system will already be built for them to link into," she says.

Cryoport's Chief Commercial Officer Mark Sawicki discussed efforts with *Pharmaceutical Technology Europe*.

Industry challenges

PTE: What is the most challenging aspect of working in cell- and gene-therapy logistics today?

Sawicki: The supply chain is far more complex than it is for monoclonal antibodies or small molecules. Regenerative medicines are patient-specific materials. You cannot simply order bulk materials and manufacture lots of product and just push those out to market. In addition, there is

a lot of variability in procuring the materials (i.e., you're typically dealing with severely immunocompromised patients, so scheduling to get them in to pull materials, to transport or manufacture them, can be an issue). In some cases, patients can get sick or there can be weather issues.

As a result, scheduling is extremely challenging, especially for autologous materials. It is a bit easier for allogenics, but, at this point, networks for transporting donor materials have not yet been fully vetted.

In addition, the manufacturing processes associated with autologous therapies are not mature. As a result, you're often extending instrumentation and processes that have been used for single-patient production to large-scale manufacturing in a commercial manufacturing environment. The interconnectivity and optimization of workflow isn't there yet. Companies such as Lonza are trying to identify automation competencies to make the process move faster and more smoothly, but, as an industry, we aren't there yet.

Finally, the manufactured materials themselves are extremely fragile and irreplaceable, but they have to be pushed back out under very rigorous timelines and exacting conditions. You don't have the latitude for deviations because they might destroy the product or even kill the patient. All of these elements create significant complexity that the industry hasn't had to deal with before.

PTE: What do these challenges mean for pharmaceutical data management and IT systems?

Sawicki: IT is a huge consideration. We are now working to integrate our individual IT systems so that information flows from one system to the next. The goal is to eliminate the need for manual intervention and reduce the risk of human error.

At Cryoport, we have also begun to use artificial intelligence (AI)-based tools to examine product workflow, the risk elements associated with moving material in, manufacturing, and moving product back out. The only way that these AI systems can be effective is by gaining access to large amounts of data, and we're now able to pull that data through our partnerships.

Potential role for AI

PTE: What is required for interconnectivity, and did you develop the AI systems in-house?

Sawicki: Systems must be nimble enough to talk to each other, so that if there is a delay during one phase of the process (e.g., during transportation, manufacturing, or administration) the information can be pushed out through the entire system so that everyone understands the ramifications of what is going on.

We hired our own data scientists to develop the AI systems, although Vinetti is working on AI systems of its own. Our use of AI is focusing on all around risk management for materials, and how we can use existing data more effectively to manage risk and improve operations, whether for storage or distribution. By the third quarter of next year, we expect to roll out a new version of our software that will include several of the AI features that we're working on now.

PTE: How do you connect to CDMOs and to the clinical side? What elements are needed in the software solution?

Sawicki: We typically connect through electronic data interchange or application programming interfaces using either a pull or push, bidirectional, or unidirectional process, from conduits that have been designed to transition or share data with clients' enterprise resource planning systems and portals, as well as Vineti's IT platform.

In some cases, elements of our IT system are replicated inside a partner's site. If a Lonza client, for example, wants to schedule distribution of an autologous product, they can go into Lonza's system, find an interface to our system, and get whatever help they need. Solutions providers such as Vineti are designing systems that are as flexible as possible to enable data to flow more easily between systems.

PTE: What led Cryoport to develop the special shipping containers?

Sawicki: The advanced therapy shipper line is a segregated, specially engineered fleet to support the distribution of clinical and commercial cell and gene products. We rolled it out to get ahead of what we anticipate future FDA [US Food and Drug Administration] regulations to look like, and focus on what we expect to become most important feature of any shipment and packaging product in this space: traceability.

Currently, two third-party foundations, the Foundation for the Accreditation of Cell Therapies and the Standards Coordinating Body for the Alliance for Regenerative Medicine, are putting together recommendations for FDA in conjunction with ISO [International Organization for Standardization] TC 276, which will establish a long-term regulatory framework for the cell and gene space.

Our goal is to use the same standards that you'd see in a GMP manufacturing environment for the equipment used to transport these materials to end users. Some are data related (e.g., for data loggers, track and trace, and geo-fencing), but the most important aspect is establishing traceability for equipment performance. We can verify and confirm the historical utilization of every piece of equipment, who used it, and where and for what. That's one of the biggest elements that is being introduced. We're also introducing a validated cleaning process for the industry that demonstrates a 99.9999% reduction in all contaminants, which brings it as close to product sterilization as possible (since it would be physically impossible to sterilize shippers and tanks out in the field). This will eliminate the risk of cross-contamination.

PTE: What are you expecting in terms of standards (e.g., for good distribution practices for the new therapies)? Are there existing standards, and what are you expecting FDA and other regulators to do?

Sawicki: Existing standards are based around International Safe Transit Association (ISTA) protocols that have been used for the past 30 years.

One liability is that these protocols incorporate a tolerance for deviation that is fine for small molecules or many biologics but could potentially render regenerative medicines unuseable.

With many cell and gene therapies, deviations from prescribed environmental conditions, even those that last for a few milliseconds, half a second, or a second, can destroy product completely. There is no visible way to tell whether the cells are still useable after an upset. You won't be able to tell if the viability of the cells has gone from 90% to 10% without doing extensive testing.

This is why full traceability is so important, so that you know how a given piece of packaging will perform each time it leaves or returns to the manufacturing facility. Traceability looks at the risk of cross contamination, at equipment performance, and at the traceability of each shipment and whether it has been subjected to any shocks or condition deviations. These data will eventually be connected to outcomes research. Say, for example, a sample has been shipped at the correct temperature, but affected by shear events along the way. It will eventually be possible to determine whether these incidents had any impact on product efficacy. If a client has dosed 13,000 patients during this time period, they would be able to examine the data and see any patterns that come up. We maintain every bit of data generated by our systems and can go back nine years and provide regulators with any data they request.

Services bottleneck

PTE: Is there a bottleneck in services for cell and gene therapy development?

Sawicki: As more products come to market, there are issues with scale. Some producers haven't had the bandwidth to keep up with demand, and this has held back their growth. Then there is the issue of viral vector manufacturing capacity and the risk of having a single supplier for the required materials. Currently, many of the materials required for GMP manufacturing of cell and gene therapies are single sourced, which is extremely risky, and manufacturers are typically smaller providers because this is a niche industry. The 800-pound gorillas aren't yet supplying reagents and other necessities because there was no market for them five years ago. However, today there is still a pipeline of 1000 clinical products, all of which use GMP starting materials from single, small provider sources.

PTE: How about scaleability?

Sawicki: This is easier to manage for autologous treatments. As you move to allogeneic products, instead of 2000 or 3000, you will be dealing with two or three million, and fleets will move from 1000 to 100,000. There's only so much existing capacity for liquid nitrogen shippers. This is also true for storage and distribution. For example, how will pharmacies do the visual inspection required when releasing allogeneic product from a liquid nitrogen tank when performing visual inspection can potentially render product unuseable? The industry must resolve many of these questions. PTE

Ask the Expert — *Contin. from page 38*

- Include a standardized CAPA.
- Remove the perception that QRM is not enforced and not part of the quality system.
- Include language that drives a holistic systems approach.
- Provide examples or a case study featuring a QRM tool.
- Expand clarity on the training and documentation needed for applying QRM.
- Clarify difference between risk evaluation and risk control.
- Define roles, particularly decision-maker roles.
- Provide more guidance on risk acceptance.
- Provide additional examples such as how to apply QRM to legacy products.
- Include examples of communication flow.
- Clarify levels of maturity for QRM in enterprise risk management.
- Offer strategies for demonstrating QRM compliance to regulators.
- Recommend how to prioritize compliance versus patient safety concerns.

Bottom line, ICH Q9 is scheduled to be revised, but the sections to be revised have yet to be identified. There is no timeline available for completion of the revision. Industry has provided feedback to representatives of the EWG on some of their thoughts on what needs to be clarified so that implementation of an effective QRM programme can be achieved.

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1. ICH, Q9 *Quality Risk Management*, Step 4 version (2005).
2. ICH, Quality Guidelines, *ICH.org*.
3. H. Gregg Claycamp, "Quality Risk Management at 15: Are QRM Resilence and Process Maturity on the Horizon," presentation at 2019 PDA Quality Week (Washington, DC, 9-10 Dec. 2019).
4. S. Ronninger, "15 Years of ICH Q9: Practical Implementation & Pitfalls," presentation at 2019 PDA Quality Week (Washington, DC, 9–10 Dec. 2019). PTE

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ICH to Revise Quality Risk Management Guideline

ICH will be taking industry comments under consideration when it revises its Q9 guideline in order to clarify QRM requirements, says Susan J. Schniepp, executive vice-president of post-approval pharma and distinguished fellow, Regulatory Compliance Associates.

Q. I have heard that the International Council for Harmonization's (ICH's) Q9 *Quality Risk Management* (1) is being revised. Do you know what the industry can expect to see with the new version of the document?

A. You have heard correctly. At the December 2019 PDA Quality Week, attendees learned that ICH Q9 Expert Working Group (EWG) Members will start working on revising the document at the 2020 spring meeting in Vancouver, Canada. The guideline was first published in 2005, and at the time of publication, it offered an overview of general quality risk management (QRM) principles including an example of a risk management lifecycle approach. In addition, the guideline provided a list of risk tools and quality system areas critical to establishing and maintaining an effective risk management programme. As stated on the ICH website, "This Guideline provides principles and examples of tools for quality risk management that can be applied to different aspects of pharmaceutical quality. These aspects include development, manufacturing, distribution, and the inspection and submission/review processes throughout the lifecycle of drug substances, drug (medicinal) products, biological and biotechnological products (including the use of raw materials, solvents, excipients, packaging and labelling materials in drug (medicinal) products, biological and biotechnological products)" (2).

Over the past 15 years, the industry has tried to implement QRM principles as a part of their quality management systems. The introduction to ICH Q9 states, "Although there are some examples of the use of QRM in the Pharma industry today, they are limited and do not represent the full contributions that risk management has to offer" (1). This statement still holds true today as citations for incomplete corrective action and preventive action (CAPA)/investigations typically ranks in the top five inspectional observations for the pharmaceutical and biopharmaceutical industries. To date, QRM implementation has used simple investigational tools to solve simple problems. In addition, the industry seems to struggle with providing data or metrics to demonstrate that information from investigation results have been used to effect continuous improvement. Speakers at the PDA conference also talked about some of the challenges the industry has faced in trying to implement QRM. These reasons include using QRM to justify actions instead of assessing risk and substituting risk-assessment tools for the QRM process (3). Other problems that seem to prevent effective implementation of QRM is using it to confirm a hypothesis or rationalize non-compliance situations (4).

Understanding the original purpose of ICH Q9 is crucial in understanding what the industry might see in terms of its revision. Current thinking, as discussed at the PDA conference, is that the EWG will focus on clarifying certain aspects of the document's concepts in an addendum to the document. Basically, the document itself will probably not be revised but instead will be enhanced by creating a partnering document that will focus more on 'how to do' and less on 'what to do' with respect to QRM. The ICH Steering Committee approved the Q9 revised concept paper in November 2019, and the EWG is expected to begin working on the revision at the ICH spring meeting. A development integrated addendum according to ICH parlance means only specific sections of the guideline will be targeted for revision but a complete revision is off the table (4).

It is unclear whether the ICH Q9 revision will become more of a QRM tutorial or whether it will clarify what needs to be achieved in order for a QRM system to be considered robust and effective. To accomplish the vision of making appropriate and acceptable risk-based decisions, QRM needs to be iterative and not a once-and-done exercise within the quality management system. An effective QRM system should implement a risk review programme to facilitate continuous improvement efforts.

At the PDA conference, attendees were given an opportunity to tell EWG representatives what they felt needed to be added or clarified in ICH Q9. They were asked to answer the following question: If you could recommend changes to ICH Q9, *Quality Risk Management*, what would you recommend? The information will be provided to the EWG representatives for consideration when they begin work enhancing the document and will be posted on the PDA Letter website. Some of the recommendations were as follows:

- Ensure that QRM is recognized by the US Food and Drug Administration (FDA) as a required quality system. Currently, the European Medicines Agency (EMA) is more likely to inspect for QRM. There should be consensus regarding more alignment between Europe and the United States on QRM.

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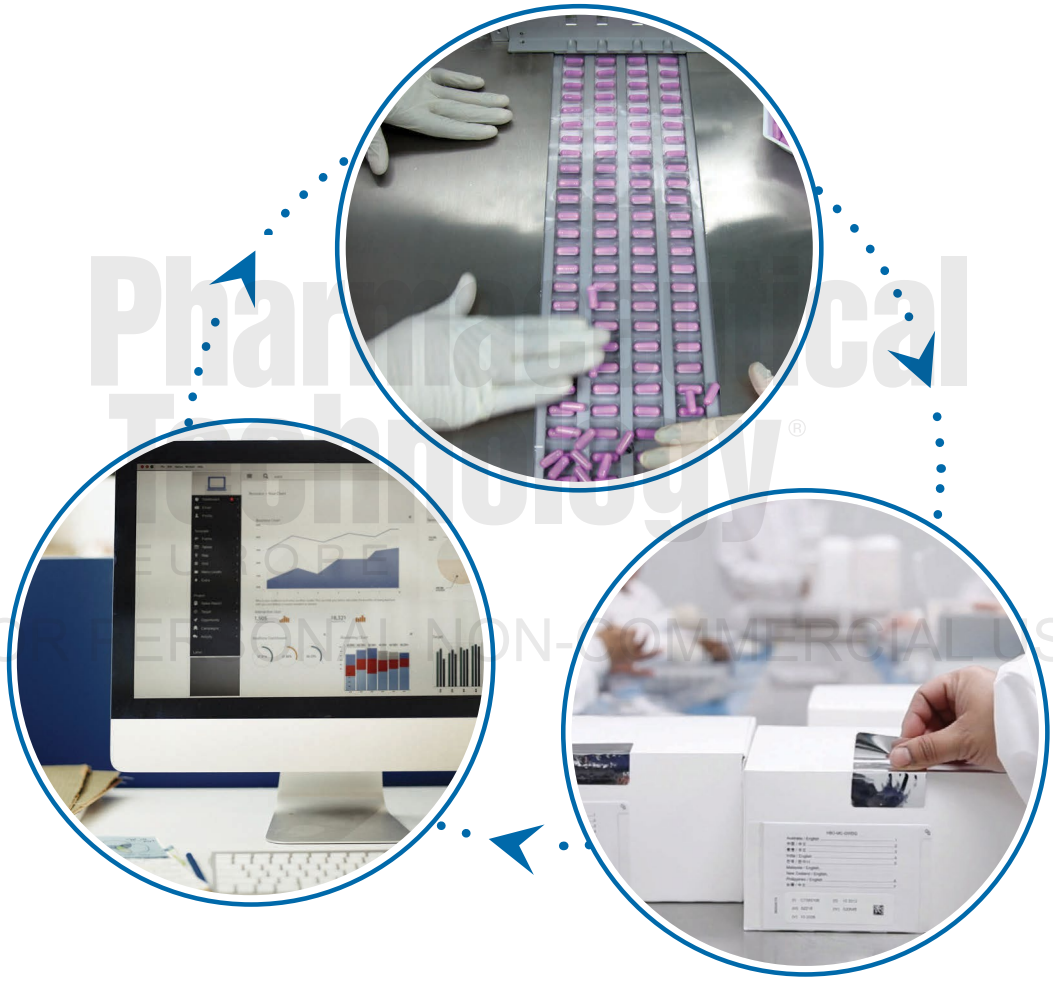

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