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News

BIO/PHARMA - MEDICAL DEVICES - COSMETICS - BIOCIDES

ddPCR in GMP product testing quantifies without calibration curve & determines absolute DNA concentration

Weihong Wang, Manager, WeihongWang@eurofinsUS.com; Jon Kauffman, Ph.D., Vice President, BioPharma Biologics, JonKauffman@eurofinsUS.com

In recent years, the number of biopharmaceutical products incorporating nucleic acids has been steadily increasing in the drug development pipeline. Some of these have made it to the market in a very visible manner such as COVID-19 vaccines that deliver messenger RNA to encode for spike protein, and gene therapies for the unmet needs of those with rare genetic disorders. Characterisation of the purity, efficacy and safety of these products is highly dependent on a technique known as Polymerase Chain Reaction (PCR). A relatively new technique for the detection and quantification of nucleic acid is digital droplet Polymerase Chain Reaction (ddPCR). In this technique, the PCR mix containing the test sample is partitioned into a large number of water-oil emulsion droplets, and PCR amplification of the target DNA sequence occurs in each individual droplet. Following PCR amplification, each droplet is assessed to determine the fraction of positive droplets in the sample. These data are analysed using Poisson statistics to determine the target DNA template concentration in the original sample.

Compared to quantitative real-time PCR (qPCR), which has become a standard methodology in most molecular biology laboratories, ddPCR has several advantages, especially in a GMP QC testing environment. First and foremost, samples can be quantified without the need for a calibration curve. In qPCR methods, a calibration curve is typically prepared from a DNA reference standard, and used to interpolate sample results. Therefore, the quality and concentration assignment of this reference standard can greatly influence the accuracy and even the validity of the reported sample results. ddPCR however, determines absolute DNA concentration through the power of statistics, thanks to the creation of tens of thousands of droplets that allow for the generation of large numbers of data points from each sample. This is particularly useful when a well characterised reference standard truly representative of the test sample is not possible, such as in the case of viral vector genome copy determination. Another advantage of ddPCR is that it is generally considered less susceptible than qPCR to PCR inhibitors that may be present in samples. This feature is especially important in the context of residuals testing, where assay sensitivity is critical. The better tolerance of ddPCR to inhibitors allows for the testing of samples without extraction, therefore greatly reducing the necessary volume of the drug substance and/or drug product allotted for testing. In the case of viral vector product testing, this can result in better preservation of products that are often produced in much smaller batch sizes compared to traditional biologics.

With the advantages discussed above, ddPCR has gained rapid momentum in QC testing laboratories. Eurofins in Lancaster, PA, has installed and validated the BioRad QX-200 ddPCR system within its molecular biology laboratories. The team has successfully performed method development, transfer, and validation projects, with a majority of them supporting viral gene therapy products. In addition to customised methods for individual clients, we are also developing generic ddPCR methods targeting consensus sequences within various viral vector backbones to support testing, including viral genome and infectious titer determination. Our in-house method validation, in conjunction with a product-specific qualification, will allow for the quick implementation of GMP testing of many sample types. For more information, visit: *www.eurofinsus.com/bpt*

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Coumarin in cosmetics: Clarifying the safety controversy

Sheryl P Denker, PhD, Senior Strategic Content Manager, SherylDenker@eurofinsUS.com; Ellen L Berg, PhD, Chief Scientific Officer, EllenBerg@EurofinsUS.com; Thierry Jolas, PhD, Study Director, ThierryJolas@eurofins.com; Eurofins Discovery.

If you're like most people, you have likely washed your hands more frequently during the COVID-19 pandemic, and you may have experienced additional dry skin as a result. Maybe you've been using more lotion, and perhaps wondering, is this product safe? In the United States, the European Union, Asia and globally, each cosmetic manufacturer is responsible for ensuring their products are safe for consumers, but the methods for determining safety risks are independent decisions. In the US, with the exception of color additives, the Food and Drug Administration (FDA) does not enforce approval authority over cosmetics, but does regulate cosmetics for safety and labelling, including for fragrances and other ingredients. Testing of individual cosmetic ingredients is, however, an active programme within the Environmental Protection Agency's (EPA) ToxCast Program. In the EU, cosmetic testing includes regulations that ban animal testing, and in Asia, according to Eurofins, toxicology testing is increasing in demand. As consumers become more health conscious and sophisticated about reading labels, companies need to emphasise the safety of ingredients that go into their products while balancing testing and regulatory guidelines.

Enter the coumarin controversy. Since the late 1950s, coumarin in food has been under scrutiny for safe upper limits related to carcinogenicity and liver toxicity in animal models. Coumarin (found naturally in tonka beans, lavender, cassia cinnamon, and sweet clover) is an aromatic lactone used by the cosmetics industry as a fragrance in perfumes, bath and shower products, lotions, and deodorants (and by the e-cigarette industry in vaping products). In 2004, although scientists and regulators determined genotoxicity was not a relevant toxicity mechanism if daily intake remained below threshold doses, concerns around coumarin continued.

In 2020, a team from the Unilever Safety and Environmental Assurance Centre published a study that leveraged Eurofins Discovery's global portfolio of safety assays to show that coumarin in personal care products has a low safety risk at exposure levels expected from application of face cream and body lotion twice daily. Taking an animal-free approach, this team used the Tier 1 SafetyScreen44™ Panel and all-human, primary cell-based assays of the BioMAP® Phenotypic Platform to assess the biological activities of coumarin relevant for a human safety risk assessment. The study, "A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products", published in Toxicological Sciences and presented to the US EPA, demonstrates the success of a major company in the cosmetics industry in assessing safety without animal testing. For more information, visit Eurofins Discovery at BioMAP Platform or SafetyScreen.

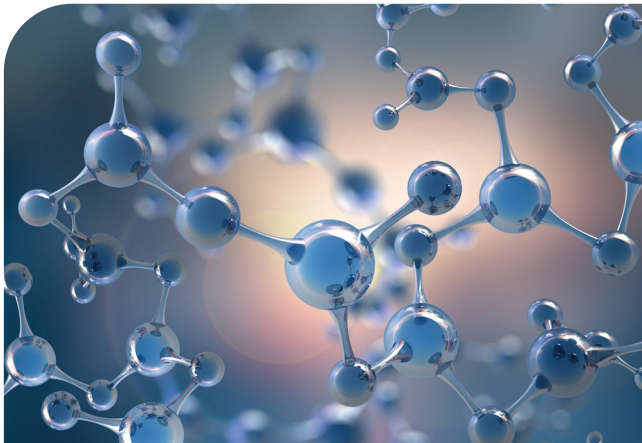
Nanomilling for bioavailability enhancement: New capabilities at Eurofins CDMO in North America

Dr. Praveen Saligram, Head of Formulations, PraveenSaligram@eurofins.com; Kevin Rosenthal, Business Head, KevinRosenthal@eurofins.com; Drug Product Operations, Eurofins CDMO Alphora Inc.

Drug discovery programmes around the world are seeing an increase in challenging lead candidates when it comes to aqueous solubility. It is widely reported that more than 90% of New Chemical Entities (NCEs) in the discovery pipeline are poorly water-soluble. Nanomilling by agitator bead mills is a versatile formulation technique for bioavailability enhancement and offers several advantages for formulators and process engineers alike. The process is relatively easy to scale-up after critical process parameters are defined, and the advantages of nanomilling include the ability to incorporate high active payloads, establishment of a continuous process during milling, and reproducibility of particle size distribution from batch-to-batch.

Nanomilling is a top-down approach towards the reduction of particle size to below the sub-micron range. Particle size reduction via nanomill is achieved by the sheer force generated during collisions between the suspended drug substance and the milling media within an enclosed chamber. The use of a wet milling media in the process increases particle surface area by several thousand fold, thereby enhancing the *in vivo* rate of solution, which leads to enhanced bioavailability of an otherwise insoluble drug substance.

Along with the nanomilling capability, Eurofins CDMO Alphora has also acquired a Zetasizer analyser that allows for sub-micron particle size and surface charge measurement. This new technology provides an ability to develop and characterise sub-micron suspensions and other colloidal dispersion systems at the North American CDMO site. Coupled with these newer capabilities are the existing bioavailability enhancement techniques of micronisation by jet milling and amorphous solid dispersion (ASD) by spray drying. This suite of advanced technologies now provides clients a full complement of formulation enabling options for poorly soluble compounds. For more information, visit: *www.eurofins.com/cdmo*



Eurofins utilises ultrasensitive methods in the battle against neurodegeneration

Kieran Kedney, Scientific Affairs Liaison, Eurofins Central Laboratory Global Scientific Affairs, KieranKedney@eurofinsUS.com

To date, there are more than 600 neurodegenerative diseases that affect the brain, spine, and peripheral nervous system. Some diseases, like Parkinson's disease affect motor functions, while others, such as Alzheimer's disease progressively destroy memory and other mental functions. Although there are numerous symptomatic treatments available, there is still no definitive cure for diseases such as Alzheimer's and Parkinson's. The World Health Organization currently estimates that over 35 million people are living with Alzheimer's disease worldwide.

Currently, Eurofins has experience with over 66 neurology trials from Phase I to Phase III. Eurofins Central Laboratory offers an extensive range of biomarker assays which are considered essential in neurodegenerative disease trials:

- *Total Tau*
- *Phospho-Tau 181*
- *Amyloid Beta 1-38*
- *Amyloid Beta 1-40*
- *Amyloid Beta 1-42*
- *Alpha-Synuclein*
- *NfL neurofilament light chain*
- *pNFH phosphorylated neurofilament heavy chain*
- *IFN gamma/IL-4 to Amyloid Pool*

These are available across various matrices, for example, cerebrospinal fluid, serum, and PBMC. Testing is available across platforms such as:

- *Various ELISA test kits*
- *Roche Elecsys ElectroChemiLuminescence immunoassays*
- *MesoScale Discovery MultiArray*
- *Simoa Quanterix SR-X*
- *ELISPOT enzyme-linked immunospot*

Multiplexed assays reduce sampling requirements, costs, testing timelines, and can offer improved assay performance. Because blood-based biomarkers are at a much lower concentration in serum and plasma than in cerebrospinal fluid, methods such as Single Molecule Array (SiMoA) can be useful due to the implementation of ultrasensitive technology to detect AD biomarkers.

Eurofins also has capabilities for inclusionary, prognostic and retrospective genetic testing for neurological disorders through Next Generation Sequencing, Whole Exome Sequencing, and a variety of other high-content analytical methods. Testing is available in predefined panels with up to 160 genes or in custom validated panels with either single or multiple SNPs or genes selected. For more information visit: *www.eurofinscentrallaboratory.com*

Analytical Quality by Design: Eurofins BPT France delivers new level of expertise for methods development

Marjorie Boscus, Study Manager, Analytical Development, marjorieboscus@eurofins.com; Alexandra Belveze, Analytical Development and Validation Projects Team Leader, alexandrabelveze@eurofins.com; Eurofins Amatsi Analytics France

Analytical method development has always been a crucial issue for biopharmaceutical industry. How can we develop fast, efficient, and robust methods to support biopharmaceutical products during their whole lifetime? From pre-clinical studies to routine analysis, Analytical Quality by Design is the answer to this challenge.

ICH Q14 (revision of ICH Q2 (R1) publication is planned for 2021 and will describe the concept of Quality by Design within the framework of analytical development. In order to provide customers with a full experience of method development and the most up-to-date support, Eurofins Amatsi Analytics, Fontenilles, France, has dedicated a team of experts since 2013 for AQbD method development.

Thanks to Design of Experiment (DoE), AQbD strategy allows screening of various critical parameters for HPLC method development. Up to 4 columns, 6 aqueous mobile phases, 2 organic solvents and 3 gradient times can be evaluated at the same time. In comparison with the classical OFAT (One Factor at A Time) approach, AQbD establishes a very wide knowledge base in a reduced time as it assesses interactions between the tested parameters. Thus the best parameters for clients' methods can be defined.

Fusion QbD® (S-Matrix) is the software used at Eurofins Amatsi Analytics to perform DoE and process the generated data; the whole statistics treatment is fast and seamless. Thanks to peak counting, Eurofins is able to determine which tested conditions are the most suitable for the method. Moreover, simulated robustness can be performed to improve method lifecycle and risk assessment. This software is directly connected to Empower3 with a UPLC system equipped at Eurofins Amatsi Analytics. The last version (9.9.0) has been acquired in 2020 and allows dynamic visualisation of the best chromatographic profile and elution conditions.

AQbD is a powerful tool to obtain robust analytical methods that will help biopharmaceutical projects to advance further and improve lifecycle management. For more information visit: *www.eurofins.fr/pharma/accueil/*

Eurofins Biomnis is helping psychiatrists to optimise the choice of antidepressants in the treatment of depression

Niamh Buckeridge, Marketing-Communications Manager, Eurofins Biomnis, NiamhBuckeridge@eurofins-biomnis.com

The challenge of depression

Depression is a common and severe illness that affects more than 264 million people worldwide (according to WHO). It can sometimes take months before the optimal treatment is found for a patient, and this long period is often marked by side effects. The subject is all the more problematic because in 15-30% of cases of major depression, patients suffer from treatment-resistant depression, and to date, there are few tools available to help these patients in therapeutic failure.

The principle of the Eurofins Biomnis ABCB1 genotyping test

A great deal of research is therefore being done to try to identify the reasons for these unequal responses to treatment, among which, the ABCB1 gene coding for a transmembrane protein, P-glycoprotein (P-gp), located in the blood-brain barrier. Research has shown that two major variations in the ABCB1 gene influence the function of P-gp and thus can predict the therapeutic effect of an antidepressant, which is a P-gp substrate. This protein, which recognises nearly 70% of antidepressants, can, depending on the variant, limit or facilitate the passage of these antidepressants into the brain. This pharmacogenetic test offered by Eurofins Biomnis therefore makes it possible to identify whether the patient is variant 1 or 2, and to help the clinician predict how their patient will respond to P-gp substrate anti-depressants.

Benefits of ABCB1 genotyping for physicians and their patients

Genotyping of the ABCB1 gene has been recommended by the Swiss Society of Anxiety Disorders and Depression (SGAD/SSAD) since 2016. The test was developed by researchers at the Max Planck Institute of Psychiatry in Munich and is offered exclusively by Eurofins Biomnis since 2018. By identifying the variant of a depressed patient, Eurofins Biomnis enables treating physicians to know the response profile of their patient, and therefore adjust their treatment accordingly. It also allows:

- *Personalised, optimised treatment enabling the prescription of the right substance at the right dosage*
- *An alternative approach for patients not responding to treatment*
- *Increased chances of finding an effective treatment quickly*
- *Higher remission rates*
- *Limiting the number of molecules to be tested*
- *Fewer side effects*

At Eurofins Biomnis, scientists work proactively with psychiatry clinics and practices all over the country to support their pursuit of finding an effective treatment for their patients as quickly as possible. The remission rate of patients who have performed this test is 21% higher than that those who have not. And as such, the Eurofins Biomnis ABCB1 genotyping test can be considered an essential tool for depressed patients on antidepressants who are in therapeutic failure and for psychiatrists dealing with this type of patient. For further information on this test, including treatment with P-gp and non P-gp substrates, please download this brochure: *Eurofins Biomnis ABCB1 Genetic Testing to Optimise the Treatment of Depression: ABCB1 Test Information*

General contact
pharma@eurofins.com

Early Clinical Development
(Full service CRO, Phases I and II, Clinical Trials Unit)
early-clinical@eurofins.com

Bioanalytics, pharmacokinetics, metabolism
bioanalysis@eurofins.com

Global Central Laboratory
clinicaltrials@eurofins.com

BioPharma Products Testing US & EU
pharma@eurofins.com

Pharma Discovery Services
discoveryservices@eurofins.com

CDMO Services
cdmo@eurofins.com

For further information & contacts in other countries please refer to our website *www.pharma.eurofins.com*.