At the Cutting-Edge of Pharma Analytics

Meet the analytical experts working to ensure the safety of medicines

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Maintaining the Analytical Edge

Working across life sciences, performance materials and healthcare divisions, Site Management Analytics is MilliporeSigma's internal solutions provider for tough analytical challenges. Here, we learn more from Christoph Saal, Director, Site-Management Analytics Healthcare, and Saskia Haehn, Manager, E&L and Packaging Materials.

Christoph, could you give us a potted history of Site Management Analytics?

So in some ways, the history of Site Management Analytics goes back to the beginning of MilliporeSigma's 350 years in business! Back then, MilliporeSigma guaranteed customers that purchased chemicals were subject to quality control for the first time. Of course, it would be virtually impossible to list all the changes and milestones since then – but it's been a road of continual improvement and innovation, founded in quality. In my 18 years at MilliporeSigma, the role of Site Management Analytics has moved away from routine quality control towards more closely supporting our research and development teams with advanced – and still high quality – analytical capabilities. Today, analytics is an integral part of R&D at MilliporeSigma; indeed, the border between the two is much less defined than it was even 10 years ago.

Today, we do remember our roots with some specialized quality control – for example, when less common techniques, such as NMR or X-ray diffraction, are needed. MilliporeSigma's business units – performance materials, life sciences and healthcare – are all subject to different regulations as the sectors deal with different products ranging from, for example, liquid crystals for displays to pharmaceutics, so they all have their own respective quality management system. On the other hand, the business is driven by a stronger need for analytics for R&D, which has led Site Management Analytics to focus on R&D activities.

The shift has also been driven by trends in miniaturization and automation. To give an example; as you bring a small molecule candidate through clinical development, you need to gain information for crystal form selection, so we do a significant amount of solid-state characterization work. Fifteen years ago, you'd perform different crystallization experiments under different conditions at perhaps a 5 g scale and then send samples to our lab for analysis. Today, the same task is done at a much smaller scale – more like 10 mg and using much more experiments. From these, we gain much more information about the behavior of a compound. The crystallization experiments and analysis of such a higher number of samples are conducted in the same laboratory to maximize efficiency and leverage automation.

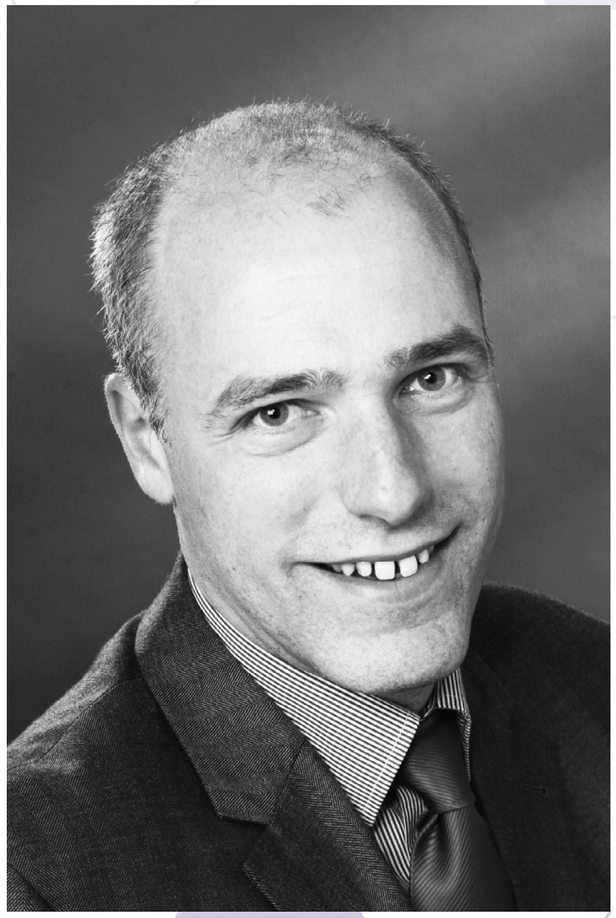
How does Site Management Analytics help its internal customers?

Our work spans three core areas across MilliporeSigma R&D – routine analytical support, project support and innovation, with the latter two taking on the prominent role in recent years. When it comes to routine support, we're responsible for making relatively simple measurements, but we use sophisticated tools, such as NMR. Here, turnaround times, cost and quality are the three drivers.

In project work, we will be tasked with solving more complex problems, which not only involves closer collaboration with our customer – the R&D team – but also other laboratories across Site Management Analytics. As an example, selecting the right crystal form of an active pharmaceutical ingredient is key when entering clinical development. Therefore, many labs doing crystallization experiments and characterizing the crystals using a broad range of analytical techniques, such as X-ray diffraction, differential scanning calorimetry, thermogravimetry, dynamic vapor sorption, measuring solubility and dissolution rate, particle size and shape, nuclear magnetic resonance, infra-red, Raman, and mass-spec, are brought together. The work is done in Site-Management Analytics involving a team of medicinal chemists, people from drug metabolism and pharmacokinetics, process development, regulatory affairs and pharmaceutical development. The answer we deliver is which crystal form should move into clinical development based on scientific consideration.

Finally, by "innovation" we really mean technology scouting and the need to consider what our customers may request or need in the years ahead. In other words, to ensure that our R&D continues to exist at the forefront, our analytical support must also be at the cutting edge. And so over the years, we've needed to add capability to support work in emerging areas – proteomics, crystal design, gene editing, for example.

I have to say that, when it comes to analytical science, remaining



competitive means building, developing and maintaining an external network; going to conferences, reading journals, making contact with external biotech companies, CROs, and universities are all key to us offering the best analytical support possible for MilliporeSigma. It matters that we are an integral part of the scientific society.

Saskia, how would you describe Site Management Analytics?
I'd probably describe us as an internal specialist CRO. We have about 230 people, including 30 PhDs and 40 engineers, with vast expertise in every aspect of analytical science, including chromatography, mass spectrometry, spectroscopy, and microscopy… everything you can imagine! We use that knowledge to support all three of MilliporeSigma's business units – life sciences, performance materials and healthcare. We are located in the headquarters in Darmstadt, but we act globally.

Could you share an example project from your lab?
Over the last 10 years, there has been growing concern about extractables and leachables (E&L) in the pharma field, and we've been involved in a number of development projects on primary packaging or process materials. I'd like to highlight one special project in my laboratory, which I think is not only of interest for MilliporeSigma's business, but also for other pharma businesses. MilliporeSigma Life Science is a global player in providing single-use systems and has a catalogue of more than 400 consumables. Recognizing the importance of E&L, MilliporeSigma launched the Emprove® program for consumables. The program collects data on the nature and concentration of extractables, in turn, helping customers with risk assessment and process design. Essentially, alongside our portfolio of single-use technologies, we offer comprehensive E&L reports, making MilliporeSigma's offerings unique. Now that we've laid the groundwork in providing this information, our customers can save time and money, because they no longer need to perform their own E&L studies.

What is the origin of the report? Well, you can probably guess that all data are generated by our experts in Site Management Analytics!

Can you offer an overview of frequently used analytical techniques?
We rely heavily on chromatography-mass spectrometry – both normal and headspace GC-MS as well as LC-MS. Both are essential for the separation and identification of inorganic extractables and the different techniques are used to cover volatile, semi-volatile and non-volatile compounds. But because of the nature of the challenge in E&L work, we have to use a number of other techniques to cover all possible entities like ion chromatography for anions and ICP-MS for elemental impurities. Invariably, more sophisticated technologies are required for the complex task of structure elucidation – which is also the most time consuming part of a project! The nature of the material also has a significant impact on the techniques required and the complexity of the task. For example, if you do an extraction of a Teflon material, the extracts will be very clean; you may only have one or two peaks to identify and quantify. But if you extract a rubber stopper? You'll get more than 50 peaks – and I'm sure you can imagine how much more time is needed…

What other challenges do you encounter in this deep analytical work?
Sample preparation is typically challenging because we have to keep the scope as wide as possible, as we do not always know what we should find in the extract. Any sample preparation task we perform can have a possible effect on the substances that are included in the extract. Moreover, we can't easily perform recovery studies because we do not know which compounds are included – and so we try to keep sample preparation to a minimum to reduce any losses of compounds of interest that may occur. Some of the compounds we are looking for require high sensitivity, which can be another challenge, demanding the best methods and instrumentation.

Finally, as guidelines for extractables testing are somewhat in development, we have to set the bar as high as we can. And that's why I'm proud of the Emprove® program – the results of which I hope will contribute to an improved regulatory landscape in some way.

What are the most rewarding aspects of your role?
The most rewarding aspect for me is knowing that I contribute to safer products for our customers – and, in turn, safer end products for their customers. I think that will always give me a good feeling! It's also very rewarding to see how much our group has grown over the years – and how much our work is appreciated.

Cutting-Edge LC-MS: Essential Technology in the Pharma Toolbox

Where would drug development be without liquid chromatography-mass spectrometry – the hyphenated technique more commonly known as LC-MS? Here, we speak with expert LC-MS user Brunhilde Guessregen, who describes its importance in impurity profiling and structure elucidation at MilliporeSigma.

What is your specific role at MilliporeSigma?

I joined MilliporeSigma in September as team leader for liquid chromatography-mass spectrometry (LC-MS) analysis for small molecules. Based on requests from MilliporeSigma chemists, we perform LC-MS analysis on APIs – specifically, structure elucidation and impurity profiling – to support pharmaceutical development within the company. But we also get involved in other specialty analyses; for example, quantifying extractables and leachables in packaging.

As is typical in the analytical world, it is challenging work; many of the structures we need to elucidate are very complex, so it can be difficult to understand what is really happening in terms of degradation and side products. Good structure elucidation requires very experienced analysts – and I personally relish the challenge of the task! And it's also a pleasure to be able to work with cutting-edge technology in our endeavours – one big advantage of working at MilliporeSigma!

Could you tell us a little more about impurity profiling – and why it's so important?

Impurity profiling is crucial to ensuring the quality and safety of the final drug product. During development, the API is well studied – and you'll know the mechanism of action and medical effects, as well as side effects. However, when synthesizing the API, side products are inevitable – and though they are typically only found in very small quantities, they can be highly active, leading to adverse effects or genotoxicity. Regulators, therefore, require in-depth reporting; you need to show what kind of impurities exist alongside your main component. Regulators mandate full characterization of your drug product, and have set specific limits for impurities; if we find an impurity within a certain range of concentrations, we need to elucidate the structure and also perform tests to assess genotoxicity. Not only is impurity profiling challenging, but it is also "high-stakes" analysis – we have to be fully confident in the results we produce, which means recruiting talented analysts and investing in the most sophisticated technology.

What technology do you rely on in your laboratory?

In terms of LC-MS (shorthand for quite a diverse range of systems), we mainly use UHPLC or direct inlet via a syringe pump coupled with high resolution MS (time-of-flight mass spectrometry from Bruker or Orbitrap from Thermo Fisher Scientific) in our lab. LC-MS has advanced significantly in recent years and it's incredible that we now have almost benchtop-sized mass spectrometers with high resolution. Compared to the "older" sector field mass spectrometers they are also far more user friendly and easier to run! I just wish that the data from different mass spectrometers could be interpreted with one software tool, which could deal with data from all mass spectrometers.

However, it is important to remember that – as with many things – more than a single tool is needed for the job. As noted, to be absolutely certain about a given structure, impurities must be isolated and fully characterized. At MilliporeSigma, we have different laboratories responsible for other specialist techniques that support our answers, such as nuclear magnetic resonance (NMR) spectroscopy and X-ray diffraction.

In short, we use whichever tool is most appropriate to the task – and, as a company, use as many tools as needed to be 100 percent confident (or as close as is humanly possible) in 100 percent of results! I don't believe that I am alone in considering "confidence" – both in the system and the results it produces – as the most important factor in analytical chemistry. We analytical chemists are driven by the desire to produce highly accurate, top quality data – and MilliporeSigma chemists depend on us to do exactly that.

What is the most important aspect of your lab's role?

The biggest advantage of this is communication – I have the opportunity to speak directly to the chemist to learn more about the API. In fact, I love discussing analytical science and techniques with the MilliporeSigma chemists we produce reports for. It's also great to be able to liaise with other labs within the company. When everything is under one roof, the system works as smoothly as

possible. Contract lab work tends to be very commercially driven, and being in-house gives us the ability to really stay on top of problems. I really think that you can have much greater confidence in your results when you have generated them in-house and as part of a team. Every morning I start my day by talking with co-workers about our pending tasks, and throughout the day I'll be involved in meetings and telephone conferences about projects. I always encourage my staff to speak about their work and challenges with structure elucidation with other MilliporeSigma scientists – that's how we can appreciate the challenges we each face, and grow as a team.

LINKS

Dissecting How Drugs Work

Frank Fischer and Sven Poetzsch have a shared goal at MilliporeSigma: to understand how medicines interact with human biology. In this pursuit, cutting-edge omics research is key.

Sven Poetzsch is Scientific Manager – Strategic Operations Bioanalytics and Biomarkers, and Frank Fischer is Laboratory Manager – Biomolecule Analytics, both at MilliporeSigma.

How do the omics fit into drug development?

Frank Fischer: The central driver of the omics concept is to achieve a deeper understanding of the biology of diseases. In very simple terms, we are looking for different patterns and the integration of information to build a better profile of a disease, and to understand what happens when it is treated. How does the medicine work? Does it have side effects? If so, how can we minimize these side effects? Many drugs end up failing clinical studies because severe side effects were not recognized in the early stages. The more you know about a molecule early on, the more you can be prepared for the future, and the better opportunity you have to optimize the treatment in the right direction – this is especially important in personalized medicine.

Sven Poetzsch: Omics technologies give us the ability to better understand what is happening on a molecular basis and should result in more targeted treatments. In addition to the aspects mentioned by Frank, such knowledge could also, in time, streamline processes and reduce the number of studies, while increasing the success rate for our drugs. A deeper understanding of the biology of the disease could also enable us to identify new targets and treatment approaches.

What are your roles at MilliporeSigma?

FF: In my lab, located in Site Management Analytics and thereby supporting a variety of different topics within MilliporeSigma, we focus on the characterization and identification of proteins and proteomes. For example, we will examine the protein sequences and check for post-translational modifications. We also do a lot of work with proteomics by setting up technologies to better understand the effects and selectivity of our compounds and how they affect the living cell. We use a range of different technologies to detect and identify protein-compound interactions within the cell, as well as try to estimate side effects.

SP: At the beginning of the year, I took on a new role within Site Management Analytics which deals with strategic topics in the context of healthcare analytics and omics technologies. We mainly deal with the quantification of compounds in biological matrices. In short, we want to know what our compounds do inside the body, and what our bodies do to the compounds! We work with cells and animal models and then, later, with samples from patients in the clinic. For a compound to be efficacious, it needs to be absorbed and distributed in the body. All compounds that enter our bodies will also eventually leave so the compound will also be metabolized and excreted. To optimize compounds in the context of efficacy and safety, it's really important to understand exactly what happens, from the compound entering the body to leaving.

We also look for metabolic biomarkers, which is where omics technologies come in. In this context, a metabolic biomarker categorizes the effect that a drug has on the body and can be a quantitative measure of an effect, how well we hit our target, and how well suited the drug is to the pathway we are targeting. Metabolic biomarkers can be also used as safety measures to identify toxic or unwanted effects; and the same applies for protein biomarkers.

How do your teams work together?

FF: What unites us, of course, is that we both want to gain a better understanding of what really happens on the cellular scale when somebody takes a medicine! We both have slightly different focus areas, but there is often overlap. I mainly focus on qualitative and semi quantitative protein analysis, such as elucidating the structure of proteins and tracking protein-compound interactions. Meanwhile, Sven focuses on small molecules and does a lot of work with metabolomics. When it comes to absolute quantification of proteins, our technologies will

overlap. For example, if a partner asks how much of the protein is within the cell then there is a connection to Sven's former lab.

SP: Mass spectrometry (MS) is a key technique for both of us. We use HPLC coupled to MS systems in different ways. Frank normally uses high resolution MS for his applications, and in my lab we use mainly tandem MS to quantify compounds. One of the obvious overlaps is that it is sometimes more beneficial with the machines in my lab to quantify compounds or even signature peptides as surrogates for the target protein.

We also collaborate on the characterization of antibody drug conjugates, where there is a combination of monoclonal antibodies that target cancer cells and cytotoxic payloads. So Frank will look into the identification and structural characterization of proteins, and I will take care of characterizing the small molecule related components, such as linkers and toxins.

What technology developments have been most important for your work?

FF: For me, it's mainly a new technology that we implemented last year called cellular thermal shift assay-mass spectrometry (CETSA-MS®), which enables hypothesis-free identification of drug to protein interactions inside the cellular environment without the use of labels. Label-free analysis is important because labels can sometimes influence the interaction of the compound and the protein. The CETSA-MS® technology also allows for off target detection, which means that we also gain important information on potential side effects.

SP: In the field of quantitative bioanalytics, the changes have not been quite as tremendous as in the world of protein analysis. There have been improvements with regards to sensitivity and speed, but overall the general concepts have not changed significantly over the past few years. In the future, I'd like to see more developments based on high-resolution MS and combined qualification/quantification strategies.

In the omics field, there is a close link to data sciences. Traditionally, science was all about a single experiment, but more and more we need to analyze a huge amount of samples in a processed way. And as we work with complex systems, you really need more than one technique to tackle the challenges. Perhaps you'll start with genomics and delve into the transcriptome, then go to the proteome, which will have an effect on the metabolites you find in your samples or in the metabolic system of cells or the body. The systems that we use in the proteomics and the metabolomics field are really powerful and create a huge amount of data, but for this to be useful we need to get the right answers out of that data and turn it into useful knowledge. Bioinformatic approaches are highly important; perhaps in the not-too-distant future we'll see great progress by artificial intelligence being applied to make sense out of increasing amounts of data.

What makes your field so exciting?

FF: When using omics technologies you always see something unexpected. And then you ask yourself: why? This ongoing puzzle is a huge inspiration and part of the reason I love the field – the new insights offered by omics always drive me to understand things further. I am also very excited about the potential for personalized medicine. The mapping of the human genome has opened up intense studies in proteomics, metabolomics and transcriptomics, and we are gaining a much deeper insight into individual differences between patients. We've always known that some drugs work better in some patients – in men, or women, or different ethnic groups – but now we are learning why. We may be able to translate this knowledge into tailor-made treatments – perhaps combination therapies – that have a higher probability of working for key patient groups.

SP: For the most part, our insight into biology is still patchy, and the fact there is still so much more to uncover makes it a very exciting field to work in. Even if you spent one hundred years in the omics field I think you'd still be discovering new things. Mass spectrometry allows us to see much deeper into biological machinery and understand some of its complexities. Ultimately, this is all about helping patients and I'm convinced that understanding biology on a molecular scale will lead to better medicines and treatment options.

All Eyes on E&Ls

Extractables and leachables require rigorous analysis to protect patient safety. Here, we speak with Saskia Haehn – an expert when it comes to diving deep into materials.

What is your role at MilliporeSigma?

I moved to MilliporeSigma around four years ago from a contract research organization, where I was working as a project manager in the field of extractables and leachables (E&L) studies. Today, as Manager, E&L and Packaging Materials at Site Management Analytics, I run a laboratory where, unsurprisingly, the primary focus is on E&L, coupled with some other packaging analysis work. Our services are available to all of MilliporeSigma's businesses, but much of our work is performing extractables studies for our life science business, which supplies single-use equipment and process materials. We also do quite a bit of work in the healthcare business.

Why is E&L so important for the industry and so fascinating for you?

E&L is a crucial topic for everyone in drug development because we all want to ensure that products and drugs are safe for patients. But from my perspective, it is also a very intellectually rewarding area to work in. Every material you examine is different, with different polymers, different uses, and the need for different extraction methods depending on the material. I am never bored! Once the first studies have been done and I've extracted the materials, the real puzzle begins. I rarely know what I am looking for, so I have to use a good number of orthogonal analytical techniques to cover everything that is in the extract.

Some confusion persists in the E&L space... Why?

Many people use the words "extractables" and "leachables" synonymously, but there's a big difference between the two. An extractable is a chemical entity, both organic and inorganic, that will extract from components of the packaging or process system into a solvent under controlled conditions (we usually experiment with harsher conditions than normal use) – we're talking about high temperatures, extended contact time, and the use of organic solvents or those with strong PH values. Extractables are important because they help us to identify the potential for leachables. Leachables are those chemical entities that migrate from components into the drug product over its lifetime, or during manufacturing.

E&Ls can theoretically come from any product contact material, such as primary packaging or process systems in contact with the drug product, but will vary depending on the activity of the material. A material like Teflon, for example, is very inert and we tend to only observe a few contaminant peaks (compounds) during our analytical tests. Natural rubber, on the other hand, can produce hundreds of peaks, all of which require thorough investigation.

Leachables can be a big risk to patient safety. In extreme cases, they can have a toxicological effect. They can also interact with the drug product, potentially reducing efficacy.

What do the regulators have to say about E&L?

E&Ls must be evaluated when determining the purity of the final product, but there are no clear regulatory guidelines on how to go about the analytical process. For primary packaging, there is USP <1663> and <1664>, but for process materials and single-use equipment, there is no real guidance – and that can be quite frustrating for the industry. Some industry working groups, such as the BioPhorum Operations Group have examined the issue and published a standardized protocol for performing E&L studies and defining thresholds for how deep your analysis has to go; however, not everybody uses this protocol, and so it can be difficult to compare results between two different vendors when trying to choose the right product.

What is MilliporeSigma's approach to E&L studies?

Actually, there is no one-size-fits-all approach for us either, because we have three business groups within MilliporeSigma that all need to deal with this topic. In the healthcare group, we must guarantee the safety of drug products and the E&L risk during production and packaging. In the life sciences group, we need to provide pharmaceutical customers with E&L information about MilliporeSigma's products, such as single-use systems. We conduct extractable studies according to standardized protocols, but if the information is insufficient then MilliporeSigma also offers BioReliance® Validation Services, which generate more E&L data in line with what the customer wants.

Generally, after performing our first extractables studies, we summarize the results, which includes the identification and quantification of extractables. Then we hand over the results to our tox department, which calculates a permitted daily exposure

limit (PDE) and compares this with results from the extractable studies. If the amount of the extractables is below the PDE, then you're safe. If it's above, then you have to perform leachables studies and look directly into the product. Depending on the results of these studies, it may be necessary to change something in the packaging or the process.

For our performance materials group, where product performance is crucial and can be influenced by leachables, we need to be very specific in what we are looking for. Some leachables can have a negative effect on performance. And sometimes the limits for these substances are much lower than the toxicology limits for drug products.

What are the main techniques used in your lab?

We rely heavily on combinations of chromatography to separate substances and mass spectrometry MS to elucidate those substances. We use both normal and head space gas chromatography GC–MS, as well as liquid chromatography LC–MS, depending on the nature (molecular weight, for example) of the compounds, but we may also need to work with a number of other analytical techniques to cover all possible entities, such as inductively coupled plasma ICP-MS for elemental impurities or ion chromatography for ionic species.

We also perform some other a analytics, such as total organic carbon and non-volatile residues, to find the total amount of extractables present in the extract. In addition, there are some material specific analytical techniques, such as the determination of nitrosamines for rubber stoppers, for example. For nitrosamines, the limits are so low that conventional techniques don't capture them, so we have to use specialist analytical techniques, such as GC-TEA for nitrosamines or LC-MS/MS for perfluorinated compounds.

What is the biggest challenge you face in your role?

The biggest challenge is handling all of the requests I receive! Our laboratory is so important for MilliporeSigma's businesses – and the topic of E&L is only becoming more crucial as time goes on. We started in 2014 with just two people in the lab. There are now eight of us and there are also more people in the specialized labs that perform more detailed structure elucidation. We have worked hard to recruit people with the right skills. A good analytical background is key, of course, as is knowledge about the product and the materials that come into contact with drug products, including pre-treatment steps such as sterilization. You need to understand how substances from the materials can be extracted and how they behave in the extract, and be able to set up a good study design. If the study set up is not adequate, then all the results you gain are useless.

Can E&Ls ever be completed eliminated?

I don't think so. I don't see E&Ls ever going away, despite the huge progress that has been made with companies really understanding their products, and developing specialized materials or coatings. Single-use products are becoming more popular in the pharma industry, but they are still relatively new and I don't think all suppliers out there at the moment can support their customers with good E&L data.

There will also always be changes in certain products. For example, if you look at resin manufacturers, they are often looking for new additives that can help them control costs. And when there is a change in the additives to the polymer, new compounds or substances will show up during extractables studies that we need to investigate.

However, I think the industry is constantly acquiring new knowledge around E&L, which can only be a good thing. In time, if we can move to more standardized approaches, then it will be a huge benefit to the industry, saving many companies a lot of time and money.