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Isotope Days 2024- Jared Kress, BSc
A Targeted LC-MS/MS Method for Routine Monitoring of Cell Culture Media Components in Biotherapeutic Processes

Andrew Percy, PhD (00:01):

The next speaker in this lineup is Jared Kress. Jared is an analytical scientist at Merck where he is responsible for developing, optimizing and implementing quantitative methodologies using targeted MS metabolomics methods. He is also involved in the development and characterization of biotherapeutic productions such as with cell culture media or CCM Today. He will present the latest work at Merck in the area of routine analysis of CCM components, utilizing a targeted L-C-M-S-M-S method and OPE labeled standards. Jared, the floor is yours.

Jared Kress (00:50):

Alright, thanks Andrew. Good morning everyone. I am Jared Kress and today I am excited to talk to you guys about my work in cell culture media characterization and how we are utilizing Cambridge Isotope Labs MSK- QREsS kit in our analysis. And it's an isotope labeled standard, which I will explain in more detail shortly. But before we dive into it, I would like to provide a little bit more background of myself. I've been at Merck now for five years supporting large molecule process characterization where I've had the opportunity to be a part of a team focusing, like Andrew said, on developing and implementing conventional and novel analytical technologies. In my role, I have a specific focus on mass spectrometry based workflows or metabolomics workflows where we kind of provide support for process robustness and understanding of our vaccines and biologics. In the talk today, I will highlight the development of this targeted triple quad mass spec assay, which we are utilizing across multiple platforms here in detecting and quantifying metabolites and cell culture. So to the next 10 to 15 minutes, we'll highlight that work, explain why we entered into this space, the type of instrumentation we are using, and how we are incorporating this isotopically labeled standard into our analysis.

02:50):

Just a brief agenda for topics today. Okay. First would like to highlight, okay, sorry. So Cambridge Isototope Labs has developed this QREsS kit which stands for the quantification retention and system suitability to enable metabolite quantification. The kit contains an isotopically labeled standard mix, which provides many uses. It can be used as a quality control check for your experiments system suitability to track performance over time. It can also be used for instrument qualification as well. The kit is comprised of a broad range of metabolic compound classes, which we find suitable for analyzing chemically defined media, which is typically used within biomanufacturing processes during the development phases. For our assay, we used this kit to assess matrix effects and extraction efficiencies with this standard. And a big part that this standard plays in our data analysis is what we call data normalization, which we base on functional and structural compound properties, which I will get into later on as well. And some of that work was highlighted in our publication, which you can find in the Journal of Chromatography A and is also linked here as well.

(04:42):

So this is a breakdown of the composition of the mix you can see. So it does come as two vials. The kit contains two vials in a lyophilized form with this kit. You also get documentation on experimental procedures, methods that you can apply in your own lab as well as those results. The concentrations are

listed here. That formulation is the result of a one milliliter reconstitution in each individual vial. It's very stable. Again, multiple uses here, which are outlined with the kit as well. Next, I'll discuss some of the key concepts with cell culture media analysis and why it is of major importance in the bio therapeutic process.

(05:45):

So let's first understand that cell culture media characterization refers to the process of analyzing and understanding the properties and components used in the growing of cells. So cell culture, media, whether chemically defined or undefined, is providing the essential nutrients, the growth factors, and the optimal environmental conditions necessary for cells to survive, proliferate and function properly. Characterizing the media is critical to ensuring the cell's health, reproducibility and overall yield at the end of the process. In addition, spent media analysis, so that's after cells have consumed or produced byproducts. That's also a vital part of upstream process development and optimization. And here are a list of a few other areas where we are employing this analysis, right? So we find it crucial to monitor lot to lot variability of vendor supplied media. So before we bring or introduce components into a process, obviously those components are vetted before introduced, but what can we do to what's a check for ensuring that there's minimal variation from lot to lot or new material.

(07:21):

So that's one area where we're using this analysis as a pre-check. With this, we can also understand cell culture dynamics over time as well. In spent media we can determine optimal feed strategy and harvest endpoints. For example, whether your process takes hours or days monitoring the output over time, maybe we can cut down on those times to incur cost savings and to speed up the process. And lastly, we can also use this to troubleshoot faulty procedures. We've been involved in many investigations where we've seen abnormal growth kinetics and one of our analysis is just one tool that the process teams can use.

(08:27):

Here I want to highlight what some of the tools are being used in the field for cell culture media analysis. For example, there's online monitoring such as ramen, where the tube where a probe is inserted into the bioreactor at line monitoring tools such as YSI and NOVA Flex are close to the shop floor bioreactors and can provide quick results. But the coverage is limited compared to other tools. But with offline tools such as N-M-R-U-P-L-C methods including LCMS methods as well, the coverage is expanded. But for these tools we have listed here, we've received feedback suggesting some inconsistencies with the data as well as reproducibility issues. So given the complex composition of chemically defined media, the need for accurate and efficient detection methods remain unmet in certain situations. So therefore, we have developed a 20 minute reverse phase LCMS MS method, which operates in both polarity modes and we can detect up to 110 metabolites with this method.

(10:06):

Here I would like to highlight the advantages of triple quadruple mass spec in cell culture media analysis. And again, I'll explain more of the instrumentation that we are using. But for those who are familiar with

triple quad mass specs, you understand the importance of high sensitivity. So providing improved detection limits, making it ideal for detecting very low levels of compounds. And you can understand why this is crucial in the field of biopharmaceuticals where there's always a need for detecting or understanding those low level concentrations of components in the media. Triple quad also provides superior selectivity. So the tandem MS MS uses two stages of mass analysis where we, the first Quadra poll is isolating those target ions. And the second one is filtering for your product ions. After fragmentation, enhancing that selectivity, it also provides quantitative accuracy. So precise quantitation is necessary. So the triple quad is widely used for this aspect, providing highly accurate and reproducible measurements. We find that it also is reducing matrix effects, so it has a better handling of complex matrices, interferences from other components. So it improves the reliability of results, especially in various types of matrix. Very versatile.

(11:54):

It has this multiple reaction monitoring technology. So it allows you to monitor multiple ions, multiple target ions simultaneously, which you can understand is very important. We want to understand how much and what is present in our media and this is a very significant tool for us as well. And lastly, lower background noise. So cleaner spectra not or limiting the questionable data, low background noise, et cetera. Leaning to clearer spectra. Here I will dive into more of the method information, which can also be found in our publication. So as I had mentioned, the CCM analysis method was developed on a SIC triple quad plus MES spectrometer, which has many key advantages which I had explained. So the way this technology works is there's a combination of three quadruples to improve that selectivity and sensitivity. So your Q1 and Q3 quadruples those regions act as mass filters where Q2 acts as your collision cell for fragmentation.

(13:16):

This setup allows for the MRM multiple reaction monitoring, making it ideal for precise quantitation of analytes. The SX 6,500 plus does have a reputation for high sensitivity detecting analytes in low concentration ranges, and there's many more technology advances within this platform, I think too many to dive into it. Here I would like to highlight the robust peak separation within the method. So samples are injected onto a phenomen kinetics at five column. It has a very diverse internal chemistry of the column. It's an excellent choice for polar and non-polar compounds, as well as other types of methods that include halogenated, conjugated or AME compounds. Other advantages include better resolution, higher efficiency, and easy method to method transferability. The LCMS method is a 20 minute method with a 15 minute gradient. You can see pretty significant separation. This is just a handful of metabolites here in the chromatogram. You can see this very specific, again, that MRM technologies allowing us to pinpoint on a specific region for a specific compound.

(15:05):

This slide here highlights the diverse or extensive coverage of media components that we can identify across multiple different types of metabolite classes such as AM amino acids, vitamins, nucleic acids, carbohydrates, and a list of other components to a very diverse panel. The next slide I'll explain. So how are we quantifying the metabolites in cell culture media? So given the nature of the components, we

thought it was necessary to find a standard mix that mimicked the diverse makeup of the media. As part of our sample prep, we are spiking in this isotopically labeled standard into the sample matrix, and we are monitoring these components listed here in the table alongside our unknown panel. Post sample acquisition data analysis involves normalizing our data to provide a relative quantitative value. To do so, we've assigned a certain isotope standard from this list to a subset or a group of analytes that are similar in retention time as well as molecular structure. So this analyte peak areas to internal standard area ratio is what we call our normalization ratio. So we provide a ratio value as a relative quantitation, but the diversity here allows us to assign specific isotopically labeled standards to a large list of unknown components.

(17:21):

So the next few slides, I will dive into some of the applications. For example, during the development of this assay, we assessed retention time reproducibility, as well as peak area reproducibility, right? So using a scheduled MRM method, it's critical that we have reproducible retention times and for the isotopic standards that were spiked into media, we monitored those peak areas over time. Just looking at stability. And for both types of analysis, we found CV values relatively low, which is showing great reproducibility for both. In this case study, we were analyzing lot to lot variability of production media that was manufactured at different sites. So we highlighted here that the amino acid data was higher in lab formulated media, which was tied to faster growth kinetic. So we could see that in lab formulation the cells were behaving and producing byproducts or growth much faster than that other sites.

(19:04):

So here we can just show that the teal color was almost doubled compared to a couple of other sites. That's based on the formulations used and things like that. On this slide, we were interested in comparing different bioreactor tanks within one facility. So samples were collected at harvest time point, which in this case was 40 hours post inoculation. On the PCA plot on the left, you can see tank number 19, which was a control in this case, showed the largest variation compared to the other tanks in the study. The components in tank 19 were lower than the rest, as shown in the heat map on the right. So tank 19 had extremely less or lower concentration of components compared to the rest. And those values on the right side or those normalized values that we are generating with the isotopically labeled standard.

Andrew Percy, PhD (20:25):

One minute to go Jared.

Jared Kress (20:27):

Okay, last slide, thankfully. So again, another showcase here. We can monitor the consumption of amino acids over time. So again, where can we see maybe a drop off or an increase at a certain point? How is that affecting overall yield and growth kinetics post-harvest? So some conclusions and path forward. This cell culture media characterization method, we're able to monitor up to 110 metabolites established on the S IEX 6,500 aspect. We're using the QREs kit, which is typically labeled standard to

provide us a normalization value. Some future work includes applying this method to build some predictive process models to support process characterization and validation studies. Other areas we're exploring, we want to expand our panel. So we're looking into high res mass spec for more of an untargeted analysis and using the QREs kit as a standard in that as well as an instrument check. So that concludes the presentation.

Andrew Percy, PhD (21:55):

Thank you very much Jared, and very enlightening talk. We appreciate you sharing your CCM characterization approach. Janet Merck in the interest of keeping this webinar on track though, we'll move forward to the next speaker and I'll ask, did you address the questions in the q and a box? There are a couple from your talk, a couple different attendees, but thank you once again.

Jared Kress (22:22):

Thank you.