Case study | 000438

Mass Spectrometry

Controlling trace elements in raw materials and cell culture media for better performance in bioproduction

Cell cultures are used for biologic production, such as active pharmaceutical ingredients, proteins or antibodies. Chinese Hamster Ovary (CHO) is one example of a cell culture that is widely used in the pharmaceutical industry to produce biotherapeutic substances through recombinant protein expression. Ensuring optimum conditions for the cell culture, as well as the composition and quality of cell culture media is crucially important to the effective production of high-quality pharmaceutical products.

Elements, particularly metals, can influence production significantly by affecting critical parameters, such as cell growth and viability, protein yield and quality. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can be used to determine elemental content in raw materials and cell culture media, both fresh and spent media. The Cell Culture and Cell Therapy division (part of Thermo Fisher Scientific) uses ICP-MS across multiple sites globally to assure correct formulation and quality of the Gibco™ cell culture media. Triple quadrupole systems, like the Thermo Scientific™ iCAP™ TQ ICP-MS, as well as single quadrupole ICP-MS systems, are operated between the sites located in Grand Island, New York, Hunt Valley, Maryland, Miami, Florida in the United States, and Inchinnan in the Unitied Kingdom.

Trace elements, in particular, transition metals can influence the performance of a cell culture significantly. Table 1 gives an overview on how common trace elements correlate with improved cell growth, productivity, and post-translational modifications.

Table 1: Common trace elements and how they interact with cell cultures[1]

Element	Function	Representative concentration
Copper	Supports mitochondrial oxidative enzymes; regulates lactate consumption	0.8–100 µM
Iron	Impacts glycosylation and microheterogeneity of product; supports cell growth and health	10–110 µM
Zinc	Enhances mAb productivity; reduces cell apoptosis progress	3–60 µM
Manganese	Improves galactosylation and reduces sialyation of product	0.4–40 µM
Molybdenum	Increases the production of physiologically active substances	0.001–0.1 µM
Selenium	Facilitates iron delivery; protects cells from oxidative stress and free radicals	0.005–0.5 µM
Vanadium	Mimics metabolic function of insulin or insulin analogs and enhances cell growth	0.1–70 µM
Cobalt	Increases the N-glycan galactosylation of product and terminal protein glycosylation; enhances productivity in chemically defined media	0–50 µM

Therefore, trace metals need to be controlled in cell culture media used for bioproduction. Typically, trace metals are incorporated in cell media in minute amounts to drive desired cell culture performance. While this process is controlled through carefully designed chemical formulation, inherent lot-to-lot and raw material supplier-based variability can lead to poor performance of the final product. Lot-to-lot consistency of the finished cell culture media is crucial and of concern with respect to consistent performance of a cell culture, especially for cell lines with known sensitivity to specific impurities. The changes in the elemental impurities of a raw material can lead to non-satisfactory performance of the medium or problems during the manufacturing process.

Typical raw materials include salts of intentionally added metals, buffers, amino acids, sugars, vitamins, nucleosides, polyamines and fatty acids. This wide variety of sample types, together with the need to accurately quantify low concentration of impurities, requires the use of triple quadrupole ICP-MS technology. Specifically, inorganic salts, like ferric citrate, zinc chloride, or nickel chloride can create a wide variety of polyatomic interferences, that render the detection of other important impurities difficult. Other components, like amino acids or sugars, may affect the response of the ICP-MS through matrix effects and again lead to a range of interferences that need to be overcome in order to obtain accurate results. A good example is cyanocobalamin (or Vitamin B12), which contains cobalt, potentially forming an interference on arsenic[2].

"The iCAP TQ ICP-MS is great, specifically the option to look at different modes of analysis," says Dr. Sam Mwilu, R&D Manager, Bioproduction Analytics in Hunt Valley, Maryland. "We need to analyze some of the raw materials [like the inorganic salts] at high concentration levels to see all the impurities. This means that polyatomic interferences can become very significant. To compare the results with different modes helps to gain confidence in the data we generate. ICP-MS is one of the most requested assays that we do, so our systems run almost every day."

Jaime Goldfuss, R&D Manager, Bioproduction Analytics in Grand Island, New York, adds "Our instrument is set up with a valve system as part of the sample introduction, which minimizes the contact of samples with the tubing and reduces analysis time. We use our instrument for several types of requests [both internal and external customers], with the primary focus on high volume throughput for spent media analysis. The method was transferred from Hunt Valley to Grand Island and worked well with very little changes from site to site."

Not only the absolute levels of impurities need to be assessed, but also lot-to-lot variability is a potential challenge. Whilst two independent lots of a given substance may have an identical purity, the overall composition may be vastly different, and hence, creating issues with the performance of a cell culture medium. For example, two lots of an inorganic salt may both be 99% pure but the remaining 1% may have different trace element profiles. Therefore, metals with a high risk of impacting the quality of a cell culture medium are monitored as part of the QC process, and all raw materials are monitored for elemental impurities before entering production.

Although there are no formal guidelines to follow currently, testing is based mostly on specific customer requirements. The United States Pharmacopeia has implemented chapters <232> and <233>, governing elemental impurities in drug products, which has been harmonized on an international level. There is no standardized testing for cell culture media yet, but under discussion now.

To help customers drive their success with bioproduction, and decrease risks due to variability in the formulation, the Gibco trace element finished good testing service was founded. A validated method was developed for concurrent monitoring of all incoming raw materials used for media formulation, as well as consistent control of impurities in finished goods. Using superior interference removal, a key feature of triple quadrupole ICP-MS systems, such as the iCAP TQ ICP-MS, is key to accurate and reliable results. In total, more than 3,000 raw materials are analyzed, and results are collected in a database for historical trending and risk assessment. Individual detailed information on the trace elemental composition of certain Gibco cell culture medium can be requested with the Certificate of Analysis for 11 trace elements, including vanadium, iron, zinc, copper or selenium. For custom formulations, predictions on the level and composition of trace metals can be made to ensure desired media attributes. For more information on the finished good testing, see reference 3.

References:

1. Ritacco, F.V., Wu, Y., Khetan, A.: Cell Culture Media for Recombinant Protein Expression in Chinese Hamster Ovary (CHO) Cells: History, Key Components, and Optimization Strategies; *Biotechnol. Prog.* **2018**, *34*(6), 1407–1426.
2. AN43403: The determination of elemental impurities in vitamin B12 supplements using triple quadrupole ICP-MS; Thermo Fisher Scientific.
3. Trace element finished-good testing for cell culture media (thermofisher.com).

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