Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-Aug-2024
Document Type: General Chapter
DocId: GUID-D7036FA9-F30F-4AFD-8754-BF80BC3A6991_2_en-US
DOI: https://doi.org/10.31003/USPNF_M17255_02_01
DOI Ref: ysber

Add the following:

▲⟨1023⟩ EVALUATION STRATEGY FOR TRACE ELEMENTS IN CELL CULTURE MEDIA USED IN THE MANUFACTURE OF RECOMBINANT THERAPEUTIC PROTEINS

Cell culture media [which includes basal cell culture media, nutrient feed, and supplements (e.g., hydrolysates)] are complex mixtures that are usually comprised of a wide variety of components, including but not limited to salts, amino acids, sugars, vitamins, and minerals. The type and amount of these individual media components are quantitatively formulated to maximize protein expression, yield, and refolding of proteins to the active state.

In addition, these media components also contain trace elements that may impact cell growth, protein expression, glycosylation patterns, and protein quality during upstream manufacturing processes. It is widely acknowledged that even small changes in these trace element concentrations in the media can have a large impact on product quality. Trace element concentration variability has been observed in both lot-to-lot and vendor-to-vendor cell culture media components. As a result, a thorough biologic elemental monitoring program for measuring and defining acceptable trace element levels is desirable to understand their impact on recombinant protein production and product quality.

This informational general chapter will discuss important topics relevant to establishing a trace element monitoring process that include:

- Strategies for identifying trace elements of interest in cell culture media
- Development of a risk assessment process for presence and concentration of trace elements in cell culture media
- Media component sample considerations and sampling procedures
- Analytical techniques and considerations important to measuring trace elements in cell culture media

A case study with a simple cell culture media is also provided to demonstrate and discuss commonly observed issues when a trace element program is implemented.

ELEMENTS OF INTEREST

Before material risk assessment and analytical method development can occur, the elements of interest must be determined. Many of the characteristics of a biologic elemental monitoring program will be specific to the therapeutic and manufacturing process in question. Select trace elements can play critical roles in production of some biotherapeutics while showing little impact in others. The elements discussed below are recommendations based upon published literature and industry experience. There may be additional elements not discussed that could potentially impact biologic upstream bioprocessing.

Identifying and measuring the variability of identified trace elements that may impact product quality is a critical step in characterizing the biologic bioprocess. If possible, process characterization work should be performed in the development phase to determine the role and concentration ranges of elements with known cell culture impact. These actions apply to anything that is added to the bioreactor as part of upstream bioprocessing, including chemically defined media (CDM) powder or liquid, hydrolysates, supplements, and individual components. Elements (such as antimony, silver, or titanium) that may be present in cell culture media but have not been reported to impact cell growth or product quality are excluded from this discussion. In addition, CDM and hydrolysates composition are often proprietary to each biopharmaceutical company, and each may have their own individual recipe suited for their product. Certain elements listed here could also be deemed as essential trace elements vital for cell growth based on each company's proprietary formulation. Elements that are often analyzed and can impact patients adversely, such as mercury, lead and arsenic, have not been shown to have historically important roles in cell culture biology. Additionally, the presence of elements such as sodium, potassium, phosphorus, and calcium in the form of salts and buffers are ubiquitous in biologic manufacturing, and therefore are also out of scope. Whether or not an element needs to be monitored in a specific process is up to the end user of the medium.

The elements recommended for characterization and monitoring are shown below. The majority of the listed elements are known to impact either cell culture performance or protein product quality. Some of the listed elements, such as vanadium and aluminum, have not been historically shown to have dramatic process impact, but their concentration variances have been noted in cell culture raw materials and are often monitored.

Table 1 lists elements that have been shown to be critical to cell viability and consistent product quality. *Table 2* lists elements that are common impurities and may impact cells. It should be noted that many elements play a role in which sufficient concentration is required for proper biologic performance, but an oversupplementation may lead to negative effects. For example, manganese is a co-factor for multiple enzymes in glycosylation pathways, yet oversupplementation can lead to decreased protein yields and cell growth. Augmented copper concentrations in culture mediums have been associated with increased lactate consumption and higher product titers, but excess free copper can generate reactive oxygen species that can be responsible for both oxidative and reductive damage to cells. For this type of variability situation, minimal concentration differences such as 100–200 nM can be large enough to cause process differences.

Table 1. Elements that have Known Impact on Cell Viability and Product Quality

Element	Potential Cell Culture Role
Cobalt	Post-translational modification effects
Copper	Required for aerobic respiration and select enzymatic pathways
Iron	Required for cell respiration and metabolism
Magnesium	DNA replication and protein synthesis
Manganese	Co-factor in glycosylation pathways
Selenium	Incorporated into antioxidant enzymes
Zinc	Stabilize cell membranes

Table 2. Elements That May Have an Impact on Product Quality

Element	Potential Cell Culture Role
Aluminum	Common raw material manufacturing contaminant
Antimony	Polyethylene terephthalate filter membrane residual catalyst contaminant
Cadmium	Can be toxic to cells
Chromium	Toxic to cells and a common stainless-steel contaminant
Molybdenum	Stainless-steel contaminant
Nickel	Stainless-steel contaminant
Titanium	Common raw material manufacturing contaminant
Vanadium	Stainless-steel contaminant

Depending on process and molecular structure of the product, certain trace elements will have a dramatically larger effect and the end user must identify which trace elements of interest are critical to their process. For example, if glycosylation is a critical quality attribute, extra attention should be paid to manganese. Alternatively, if charge variant species is a critical quality attribute, then it is recommended to monitor copper and iron. It is essential to scan the literature for known product quality impact and if possible, conduct lab experimentation with small scale bioreactors to test individual cell culture process sensitivity to different elemental concentrations. Further risk assessment of individual raw materials that could potentially cause contamination of certain elements is also recommended to understand the supply chain, the geographical region of mining, vendor processes, or purification changes. All these supply chain risks should be considered to build comprehensive risk assessment and mitigation strategies. Do note that qualification work of the analytical method should be performed to verify that the method developed produces accurate, precise, and repeatable results as explained later in this chapter. There may be interference issues in the inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectroscopy (ICP-OES) instrument with the addition of each new element, which can complicate method development and qualification.

RISK ASSESSMENT

In order to ensure that the critical attributes of the biological product remain consistent, a risk management process should be maintained throughout the product life cycle recommended to be based on the principles of the International Council for Harmonization (ICH) guideline on Quality Risk Management (Q9) (*1*). A risk assessment is a systematic process of organizing information to support a risk decision to be made within a risk management process. To frame and inform the overall risk assessment, it is essential to understand what can go wrong, the probability that it will go wrong, and the consequences (severity). A properly conducted risk assessment can save time and resources, while supporting process consistency and product quality. An effective quality risk management approach can further ensure the high quality of the drug product being delivered to the patient by providing a proactive means to identify and control potential quality issues during development and manufacturing.

In the case of cell culture media, there is a need to focus the risk assessment on the potential impact that raw materials in the media may have on the biological molecule, or on the cell culture. It is not uncommon for metals to—directly or indirectly—make a positive contribution to the media as part of the final media formulation; however, that positive impact can be within a narrow concentration range. The risk assessment should take this into consideration.

Risk assessments are not a "one and done" process but are routinely done as part of the life-cycle management of a molecule (2). An example of a good place to start to develop the risk assessment is with the Ishikawa, or "fishbone" diagram. Such diagrams can help to bring into focus areas where a potential risk may exist.

Risk assessment tools, such as failure modes and effects analysis (FMEA) or other risk ranking tools, can be used to evaluate material risk and drive required control actions. In addition, a good relationship with raw material suppliers should be established and maintained via effective communication tools, in order to ensure process changes are thoroughly documented and evaluated for possible impact to the quality of the cell culture media. Some risk factors for trace elements in cell culture materials are listed in *Table 3*.

Table 3. Potential Trace Element Risk Factors

Potential Risk Factors	Considerations	Common Examples
Source of material	• Mined material, such as various salts, can have higher levels or variability of trace metal impurities. • Animal- and plant-sourced materials may have higher variability of trace metal impurities. • Synthetic source materials tend to have lower variability but should be evaluated based on the chemistry of the synthesis and manufacturing process.	• Materials such as iron salts have historically been more challenging with respect to trace element impurities because they are mined materials.
Source location change	• Changes in location source may impact trace element variability.	• Change in mining sites for raw material
Grade of material	• Grade of material (e.g., compendial, ACS, technical) indicates degree of purity and variability of impurities.	• Testing specifications vary based on grade of material.
Manufacturing process	• The manufacturing process of the raw materials (synthesis, fermentation, biosynthesis, extraction, and/or purification) can impact the trace element impurities. ◦ When troubleshooting manufacturing processes, equipment, reagents, or metal catalysts used should also be considered. • Consider using single-use materials when possible. • Highly complex or less well-controlled raw material manufacturing processes can lead to higher variability.	• Corrosion of stainless-steel tanks can be a source of trace element variability.
Change to manufacturing process or source	• Vendor changes in the manufacturing or purification process for a raw material may impact the trace element impurity profile.	• Columns for purification being updated with new technology (e.g., diatomaceous earth to synthetic chromatography resins) or the

Potential Risk Factors	Considerations	Common Examples
	- Change control notifications from vendors should be carefully reviewed and should include data from a minimum of 3 lots.	vendor moving to a new manufacturing site. - Transfer to a new manufacturing location may impact processing materials such as the source of water within the manufacturing process.
Material complexity	- Consider if the material is single component, multicomponent, chemically defined, or chemically undefined.	- More complex chemically undefined materials (such as hydrolysates) may introduce more variability.
Quantity used	- The amount of the ingredient used in the cell culture affects the potential risk contribution from that ingredient.	—
Supply chain	- Assess the effectiveness of the supplier's Quality Management System, including a change notification program.	- Ineffective communication of change control can result in changes that may impact the trace element profile.

The risk assessment assists in identifying potential high-risk materials that need to be monitored for trace elements. The assessment is performed for single component and multicomponent media used in cell culture. For multicomponent media, the risk assessment may be performed on each component. Alternatively, the components may be categorized into groups, such as amino acids, metal salts, hydrolysates, and vitamins. The risk assessment should then be performed on each category. In many instances it may not be possible to assess the impact of components such as trace elements as this would require complete access to the medium formulation from a vendor, and this is typically not possible. The risk assessment may be conducted by the drug product manufacturer, the supplier, or a collaboration between the drug product manufacturer and the supplier. The outcome of the risk assessment informs which materials require monitoring and the monitoring frequency.

SAMPLE CONSIDERATIONS

Components of cell culture media are shown in *Table 4*. The careful preparation of cell culture media or media components for analysis of elemental impurities by ICP-MS is critical for achieving precise, accurate, reproducible results. The samples must be handled in a clean and controlled environment with appropriate equipment and storage containers to avoid environmental contamination. High purity materials and reagents suitable for mass spectrometry are required.

Table 4. Components of Cell Culture Media

Sample Type	Definition	Examples
Basal cell culture medium	A complex mixture of components necessary to grow and maintain cells in vitro. A cell culture medium may also be differentiated as a growth or a production medium, depending on the stage of the cell culture process and the medium's main purpose (optimizing either growth or production of the protein of interest).	Classical formulations such as Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute Medium (RPMI)
Feed	Also known as nutrient feed, a complex mixture of components usually prepared at significantly higher concentrations than cell culture media, to replenish consumed components during a cell culture.	10-fold concentrated DMEM might be considered a feed for a DMEM cell culture media

Sample Type	Definition	Examples
Supplement	An individual component, or a mixture of components added separately to cell culture medium usually before or during cell culture. Supplements can be added to trigger a specific action or to provide specific nutrients to cell culture. In addition, components may be supplemented due to instability.	Amino acids, dipeptides, carbohydrates, peptones, hydrolysates, recombinant insulin, methotrexate (MTX), methionine sulfoximine (MSX)

Sampling Procedures

It is important to ensure the homogeneity of samples for trace elements analysis so that the results obtained are representative of the entire lot or batch. It is recommended to follow ICH guidelines for sampling. Samples must be representative of the entire batch of material. If homogeneity is of concern, this should be resolved before continuing with testing.

A sampling plan should be developed to determine frequency of sampling, sampling sizes, and acceptance limits, and is typically based on statistical criteria. An often cited standard is the World Health Organization (WHO) guidelines for sampling of pharmaceutical products and related materials (*3*). The sampling plan should consider the specific attributes being measured (in this case, trace elements impurities), and the risks associated with accepting a defective lot. Knowledge of the material and process should be included in the risk assessment for the sampling plan.

For final blends and media components, sampling should be designed to satisfy the inherent variability of a complex chemically defined (CD) powder mixture. The number of samples taken per lot depends on the size of the lot and the criticality of the component.

Raw materials are assumed to be uniform and should be prepared at the optimal concentrations to meet instruments' total dissolved solids (TDS) requirements. Concentrations may also be determined by solubility and desired limits of detection of elements of interest.

Sometimes samples can be composited prior to testing. For example, if a single lot of material is separated into different packaging containers, a single sample can be generated for testing by compositing subsamples from the unique packaging containers. Considerations with this approach should include mixing and homogeneity of the composite. The composited sample should be from a single manufacturing batch.

Sample Types

The two main sample types are cell culture media and individually added raw materials used to prepare final cell culture medium or added directly to the cell culture. The testing procedures may vary depending on the sample type. A single testing procedure can often be used for media samples since most media are soluble in water or have known dissolution procedures. However, procedures for individual raw materials may vary depending on the material.

Some materials may be considered high risk and therefore require special attention (*4*). For example, some materials may be known to frequently have the same recurring elemental impurities. This is often related to how the material is produced, processed, and/or stored. Further, some materials may be used at different concentrations in various media formulations and thus carry different levels of impurities and associated risk. It may be beneficial to identify these high-risk and low-risk materials so that procedures may be adjusted to mitigate the risk accordingly.

Sample Size

Sampling size must be determined such that the compound can be completely dissolved and remain within the requirements of total dissolved solids for the ICP-MS. Sample size will vary depending on the composition of the sample, prior knowledge of common impurities, and the purpose of testing. For example, with samples of high sugar content, a sample size of approximately 0.5 g may be sufficient and will help keep the carbon content within acceptable range for the ICP-MS. When testing for low level impurities it may be advisable to use a minimum 2.0 g sample.

ANALYTICAL TECHNIQUES

Chemistry

Some materials may react with common ICP-MS diluents. For example, silver nitrate precipitates if added to a diluent containing chloride. Tin chloride, while fully soluble in water, may hydrolyze to form an insoluble basic salt. Precipitation reactions such as these must be known and avoided for each material. Additionally, some elements may be more volatile, unstable, or prone to stick to container or tubing walls than others. Procedures must be adjusted to eliminate errors related to the chemistry of each material.

Analytical Instrumentation

The selection of appropriate analytical instrumentation for the quantitative measurement of trace elements in cell culture media is highly dependent on several factors, such as specific analyte elements, number of target analytes, target concentration of each element, required method accuracy and precision, specific media composition, required analytical throughput, and experience level of the analytical staff.

Quadrupole-based inductively coupled plasma mass spectrometry (ICP-Q-MS) will likely be applied most frequently for quantifying trace elements in culture media. It is an ultra-trace, multi-element capable class of instrumentation with a very broad dynamic range. As a technique, this class of analytical systems have reached a high level of maturity, their analytical issues have been well defined and addressed, they are relatively commonplace, and there are many experienced ICP mass spectrometrists now well-versed in ICP-Q-MS (5).

However, other instrumentation techniques are present within the industry and can be appropriate for the quantification of trace elements in some circumstances. ICP-OES, graphic furnace atomic absorption spectroscopy (GFAAS), hydride generation atomic absorption spectroscopy, or x-ray fluorescence spectroscopy (XRF) are capable of detecting and characterizing trace elements in cell culture raw materials, each with their own set of limitations. For the purpose of this chapter, ICP-MS is seen as the most versatile and sensitive analytical technique for elemental characterization and will be the focus of this discussion on analytical procedures.

Sample Preparation

Forms of sample preparation include neat, direct aqueous solution and indirect solution. The selection of the appropriate sample preparation is dependent on the material under test and is the responsibility of the analyst. See *Table 5* below:

Table 5

Sample Type	Recommended Preparation[a]
Neat	Use undiluted liquid sample
Direct aqueous solution	Use when sample is soluble in aqueous solvent
Indirect solution	When sample is not soluble in aqueous or organic solvent, use closed vessel digestion procedure
Solid sample	Completely dissolve in appropriate solvent and dilute as necessary
Liquid sample	Dilute in appropriate solvent as necessary
Samples containing organic components	Dilute in appropriate solvent and heat digest until clear

[a] See *Plasma Spectrochemistry ⟨730⟩* and *Plasma Spectrochemistry—Theory and Practice ⟨1730⟩* for detailed information.

MATERIALS AND REAGENTS

Sample container: It is recommended that samples be stored in acid washed or low heavy metals verified plastic containers (single use or disposable).

Deionized water: 18 MΩ.cm ultra-high purity water for dilution

Acids: High purity mass spectrometry grade (examples—nitric acid, hydrochloric acid, sulfuric acid)

Calibration standards: Traceable to a standard-setting organization

Internal standards: Certified ICP-MS grade reference materials

Sample handling: To minimize contamination, samples can be handled and prepared in a chemical fume hood. A trace-element-specific fume hood with a laminar flow air curtain can also be used. The hood must be clean and free of potential environmental contaminants. It is recommended to have a dedicated area for trace element sample preparation. Potential sources of environmental contaminants include air vents, ceiling tiles, paper towels, gloves, and glassware. Consumables such as tubing, pipette tips, and reagents are another source of contamination.

A laboratory reagent blank containing all the components used in sample preparation except the sample should be prepared and analyzed in parallel with all samples.

INTERNAL STANDARDIZATION AND SAMPLE DILUTION CONSIDERATIONS

Culture media are a complex, multi-component mixture of carbohydrates, salts, nucleic acids, amino acids, vitamins, and nutritional trace elements. It is helpful to the success of an ICP-MS analysis to have knowledge of the specific media components and their concentrations.

In most cases, the culture media is directly dissolved with a dilute acid diluent. The media will be highly water soluble and performing a total digestion of the media will not remove the inorganic components from the test solution matrix.

Generally, the strategy for ICP-MS sample dissolution is that the total percentage of dissolved solids is not more than 0.2% (the amount of total solute in grams per 100 mL of diluent). Inorganic salts in particular can affect the accuracy of an ICP-MS experiment. Ideally, the total amount of sodium introduced to the mass spectrometer, for example, should not exceed 1000 µg/mL (ppm). The presence of ppm levels of sodium (or elements that are readily ionized) affects the ICP-MS experiment in three ways:

- The plasma ion source becomes more conductive, which renders it significantly cooler than "normal" plasma temperature when introducing a clean working standard matrix such a 1% (v/v) nitric acid. This in turn lowers the fraction of ionized elemental atoms (see *Table 2*). The higher the ionization potential of the element, the more pronounced is this affect.
- The presence of the conductive ions in the ion beam changes the influence of the pole bias in the analytical quadrupole. This can significantly shift the count-rate of the lower mass ions upwards relative to the count-rate of the high mass ions.
- There is an overall loss of response over time due to the deposition of the sodium ions onto the surface of the plasma or vacuum region interface.

For all analyses, it is particularly important to apply the correct internal standard to the analyte. It is recommended that an internal standard element or elements are added to all calibration standard and sample solutions to act as reference intensities. The intensity of the internal standard mass is compared to the same signal from a chosen reference point such as the initial blank solution. Multiple internal standard elements may be needed depending upon the sample matrix, polyatomic interference, and elements of interest. Internal standard selection should be addressed during the method characterization experiments to establish suitable accuracy. In many instances, the most important consideration is matching the mass range of the analyte elements with that of the internal standards, but the effect of the salt ions on the fraction ionized needs to be considered. During the course of internal standard selection, one should be vigilant in selecting elements that do not have overlapping isotopic masses and that the test solutions do not contain the internal standard element.

From the ionization potential data presented in *Table 6*, note that there are two elements—selenium and zinc—that have ionization potentials approaching 10 eV. Correspondingly, there are two internal standard elements—beryllium and tellurium—that also approach 10 eV. The majority of the other trace element and internal standard elements have ionization potentials of 8 eV and less.

Table 6. Media Trace Element and Internal Standard Element Ionization Potentials (4)

Element	Analytical Mass (AMU)	First Ionization Potential (eV)	Fraction Ionized at 7500° (%)
Aluminum	27	<7	98
Antimony	121, 123	7–8	78
Beryllium[a]	9	9–10	75
Bismuth[a]	209	7–8	92
Cadmium	111, 114	9–10	85
Cobalt	59	7–8	92
Chromium	52, 53	<7	98
Copper	63, 65	7–8	90
Gallium[a]	69	<7	98
Germanium[a]	72	7–8	90
Indium[a]	115	<7	99
Iron	56, 57	7–8	96
Lutetium[a]	175	<7	99
Magnesium	24, 25, 26	7–8	98
Manganese	55	7–8	95
Molybdenum	92, 95, 96, 98	7–8	98
Nickel	60	7–8	91
Scandium[a]	45	<7	100
Selenium	77, 78, 80, 82	9–10	33
Tin	118, 120	7–8	96
Terbium[a]	159	<7	99
Tellurium[a,b]	126, 128, 130	9–10	66
Titanium	47	<7	99

Element	Analytical Mass (AMU)	First Ionization Potential (eV)	Fraction Ionized at 7500° (%)
Uranium[a]	238	<7	100
Vanadium	51	<7	99
Yttrium[a]	89	<7	98
Zinc	64, 66	9–10	78

[a] Internal standard element.

[b] Isotopes of tellurium have isobaric interferences with tin and antimony.

MATRIX EFFECTS

ICP-MS is a matrix dependent technique. Matrices influence the nebulization and ionization efficiencies, which are proportional to the signal intensity. Hence, it is important to match the elemental matrix components of the calibration standards and samples to the greatest extent possible, irrespective of the sample preparation procedure.

The remedies for maintaining accuracy of trace element measurements in the salt-containing media test solutions are to dilute the sample matrix, apply appropriate internal standardization, and/or matrix match the working calibration standard diluent with the media test solutions.

Carbon from organic matrices present in cell culture media is likely to cause plasma enhancement for some elements such as arsenic and selenium (6). To counteract the carbon enhancement effects, a carbon source such as alcohol may be added to the diluent to ensure both standard and sample have similar matrix composition.

ISOBARIC INTERFERENCES

ICP-Q-MS instruments are unit-resolution mass spectrometers. They are therefore subject to compromised specificity from well-characterized direct isotope mass interferences (isobaric) and polyatomic mass interferences. The term "polyatomic" applies specifically to species of ions formed from constituent ions in the first vacuum region of the ICP mass spectrometer. A non-comprehensive list of representative trace elements and corresponding interferences are listed in *Table 7*.

Table 7. Unit-Resolution Isobaric and Polyatomic Interferences

Element	Analytical Isotopes (Isobaric and Polyatomic Interferences)	Comments
Aluminum	27 ($^{13}C^{14}N$, $^{1}H^{12}C^{14}N$)	Challenging at low concentration
Antimony	121, 123	Impurity; no significant interferences
Cadmium	111 (^{16095} Mo), 114 (^{114}Sn)	Impurity; kinetic energy discrimination to attenuate ^{16095}Mo; interelement correction for ^{114}Sn
Cobalt	59 ($^{1}H^{58}Ni$)	Nickel background from plasma or vacuum interface
Chromium	52 ($^{12}C^{40}Ar$, $^{12}C^{40}Ca$), 53 ($^{16}O^{37}Cl$, $^{1}H^{12}C^{40}Ar$, $^{1}H^{12}C^{40}Ca$, $^{13}C^{40}Ar$, $^{13}C^{40}Ca$)	Constituent or impurity
Copper	63 ($^{40}Ar^{23}Na$), 65 ($^{40}Ar^{25}Mg$)	Sodium, magnesium present in media
Iron	56 ($^{40}Ar^{16}O$, $^{40}Ca^{16}O$), 57 ($^{40}Ar^{16}O^{1}H$, $^{40}Ca^{16}O^{1}H$)	Can be present in high concentrations or measured as an impurity
Magnesium	24 ($^{12}C^{12}C$, $^{1}H^{23}Na$), 25 ($^{12}C^{13}C$, $^{12}C^{12}C^{1}H$), 26 ($^{12}C^{14}N$, $^{12}C^{13}C^{1}H$)	Commonly a constituent at non-trace concentrations
Manganese	55 ($^{16}O^{39}K$)	Potassium present in media
Molybdenum	92, 95, 96, 98	No significant interferences

Element	Analytical Isotopes (Isobaric and Polyatomic Interferences)	Comments
Nickel	58 ($^{58}\mathrm{Fe}$), 60 ($^{1}\mathrm{H}^{59}\mathrm{Co}$, $^{12}\mathrm{C}^{48}\mathrm{Ti}$)	Nickel background from plasma or vacuum interface
Selenium	77 ($^{40}\mathrm{Ar}^{37}\mathrm{Cl}$, $^{40}\mathrm{Ca}^{37}\mathrm{Cl}$), 78 ($^{78}\mathrm{Kr}$, $^{1}\mathrm{H}^{40}\mathrm{Ar}^{37}\mathrm{Cl}$, $^{14}\mathrm{N}^{64}\mathrm{Zn}$, $^{12}\mathrm{C}^{66}\mathrm{Zn}$), 80 ($^{80}\mathrm{Kr}$, $^{40}\mathrm{Ar}^{40}\mathrm{Ar}$, $^{40}\mathrm{Ca}^{40}\mathrm{Ar}$), 82 ($^{82}\mathrm{Kr}$, $^{16}\mathrm{O}^{66}\mathrm{Zn}$)	Krypton present in liquid argon (78 Kr: 0.4%, 80Kr: 2.3%, 82Kr: 12%)
Tin	116 ($^{116}\mathrm{Cd}$, $^{1}\mathrm{H}^{115}\mathrm{In}$), 118, 120	Constituent or impurity
Titanium	46 ($^{45}\mathrm{Sc}^{1}\mathrm{H}$), 47 ($^{16}\mathrm{O}^{31}\mathrm{P}$, $^{12}\mathrm{C}^{35}\mathrm{Cl}$), 48 ($^{48}\mathrm{Ca}$), 50 ($^{50}\mathrm{Cr}$)	Impurity; 48 and 50 isotopes not generally available for media testing
Vanadium	51 ($^{16}\mathrm{O}^{35}\mathrm{Cl}$, $^{14}\mathrm{N}^{37}\mathrm{Cl}$)	Chlorine present in media
Zinc	64 ($^{32}\mathrm{S}^{32}\mathrm{S}$), 66 ($^{32}\mathrm{S}^{34}\mathrm{S}$)	Sulfur present in media

Polyatomic interferences are attenuated using collision or reaction cell(s) technology. Collision or reaction cells are components of the mass spectrometer's ion lens assembly. They are essentially multi-pole ion guides that are filled with inert (helium, hydrogen) or reactive (oxygen, ammonia) gas. If inert gas is applied, the mode of acquisition is kinetic energy discrimination (KED) mode. If a reactive gas is applied, the mode of acquisition is dynamic reaction cell (DRC) mode. The objective is to discriminate the polyatomic ion from the element ion using physical or chemical attributes of the polyatomic ion to either reduce mass or render it neutral. If the ion lens assembly is configured with two ion guides plus a quadrupole for mass discrimination, it is called a "triple quad" configuration. A triple quad spectrometer can perform a KED experiment in one cell and then a DRC experiment in the second cell. Typically, this is done to form a stable oxide of an element and move the ion to a mass located 16 units higher. The selenium isotope at 80 amu, for example, would be measured as the oxide at 96 amu, and would be clear of the interferences listed for 80Se in *Table 7*.

In general, charging the cell with a gas will markedly improve the signal-to-background ratio of the measurement but will generally lower the count-rate of all analyte measurements. Modern systems compensate for this by allowing rapid acquisition mode changes during an analytical run, or acquisition modes to be changed and the samples analyzed sequentially.

For direct inter-element isobaric interferences, the remedy is to avoid measuring that mass, using an inter-element correction, or implementing a high-resolution mass spectrometer. A high-resolution mass spectrometer can baseline resolve the element isotope from most polyatomic and isobaric interferences.

METHOD CHARACTERIZATION

After determining appropriate standard preparation, sample dilution, and instrument setup parameters, method characterization experiments should be performed to understand and qualify the capabilities of the analytical technique. Common analytical figures of merit assessed include linearity of calibration standards, determination of limits of detection and of quantitation, accuracy, precision, and robustness. The use of sample matrix spiked solutions is critical to accurately address the method's capabilities in a realistic scenario. *Plasma Spectrochemistry ⟨730⟩* and *Validation of Compendial Procedures ⟨1225⟩* provide guidance on procedures for trace element method characterization including a discussion on analytical performance requirement differences for impurity and assay analyses. These include the number of unspiked and spiked samples to prepare in addition to proposed criteria that should be considered.

The requirements for these experiments should be entirely based on the specific use case for the method. If the method is characterizing raw materials for a biologic process sensitive to nanomolar shifts in elemental concentrations, then stringent requirements must be placed on the accuracy and precision of the instrument at minimal concentrations. However, if the method serves to only characterize broader changes over time, higher spike concentrations can be used with a wider acceptability limit.

CASE STUDY

Example of Trace Metal Determination in Media Analytical Process

To demonstrate the applicability of this chapter, a fictitious media, USP-1, is selected for analysis. This example will serve to illustrate how trace metal content is evaluated by ICP-MS. Component and trace element concentrations are taken from commercially available media (7) and from Kenerson's patented cell culture medium containing transition metals or trace elements (8).

Table 8. Principal Component Concentrations in Example Media (6)

Component	Concentration (g/L)	Concentration (g/100 mL or gram %)
Glucose	150	15

Component	Concentration (g/L)	Concentration (g/100 mL or gram %)
Sodium chloride	7	0.7
Potassium phosphate dibasic	7	0.7
Amino acids (total)	0.9	0.09
Total	165	17

Principle component concentrations in an example media are summarized in *Table 8*. For the purposes of demonstration, other components like vitamins, surfactants, and other organic nutrients are not considered. Depending on media composition, it may be necessary to identify and mitigate impact of other components.

The trace elements included in *Table 9* are also present in USP-1. The objective is to measure and confirm the actual trace element concentration in this media. The assumption is that USP-1 is solubilized, and the concentrations are presented in units of micrograms per liter. Do note that ICP-MS analysis only provides the concentration of metal and not for the salt form that is used in the media. The analysts must ensure correlation of the trace metal concentration to the salt form version of that metal used in the media formulation.

Table 9. Trace Element Concentrations in Example Media (*7*)

Trace Metal Source	Concentration (M)	Molecular Weight (g/mole)	Molecular Concentration (µg/L)	Element Concentration (µg/L)
Ammonium molybdate	4.39×10^{-10}	196	0.0858	0.0420 (Mo)
Chromium sulfate · $15H_2O$	7.54×10^{-11}	662.4	0.0499	0.00392 (Cr)
Cobalt chloride · $6H_2O$	1.82×10^{-9}	237.93	0.433	0.107 (Co)
Cupric chloride · $2H_2O$	2.06×10^{-8}	170.48	3.51	1.31 (Cu)
Cupric sulfate · $5H_2O$	1.81×10^{-9}	249.69	0.452	0.115 (Cu)
Ferric ammonium citrate	2.35×10^{-3}	261.98	615653	131000 (Fe)
Manganese sulfate · H_2O	8.99×10^{-11}	169.02	0.0152	0.00494 (Mn)
Nickel sulfate · $6H_2O$	4.54×10^{-11}	262.85	0.0119	0.00266 (Ni)
Sodium meta vanadate	4.59×10^{-10}	121.029	0.0556	0.0234 (V)
Sodium selenite	9.08×10^{-9}	172.95	1.57	0.717 (Se)
Stannous chloride · $2H_2O$	4.49×10^{-11}	225.65	0.0101	0.00533 (Sn)
Zinc chloride	1.84×10^{-7}	136.286	25.1	12.0 (Zn)

Please note that the common addition of cyanocobalamin (vitamin B12) will also contribute to the total cobalt concentration of the media. To simplify the example, the cyanocobalamin concentration is not considered.

In this example, two things are immediately apparent to the analyst: the trace element concentrations are low except for iron, and the media has relatively high concentrations of carbohydrates, amino acids, and inorganic salts, which are contributing significant concentrations of sodium and potassium in this example media.

Following best practices, the total dissolved solids of a test solution introduced to an ICP-MS mass spectrometer should be in the range of 0.1–0.2 g/100 mL or 0.1%–0.2%. As our media has a total dissolved solids content of approximately 17 g/100 mL, a convenient dilution of 100-fold would be in that range. The impact on our trace element concentration in our diluted sample is presented in *Table 10* below.

Table 10. Trace Element Concentrations in 100-Fold Dilution of Example Media

Trace Element	Media Trace Element Concentration (µg/L)	Media Diluted 100-Fold Trace Element Concentration (µg/L)	Media Diluted 100-Fold Trace Element Concentration (ng/L)
Cobalt	0.107	0.00107	1.07
Chromium	0.00392	0.0000392	0.0392
Copper (chloride)	1.31	0.0131	13.1
Copper (sulfate)	0.115	0.00115	1.15
Iron	131000	1310	—
Manganese	0.00494	0.0000494	0.0494
Molybdenum	0.0420	0.0004203	0.420
Nickel	0.00266	0.0000266	0.0266
Selenium	0.717	0.00717	7.17
Tin	0.00533	0.0000533	0.0533
Vanadium	0.0234	0.0002338	0.234
Zinc	12.0	0.120	120

Clearly these element concentrations, except for iron, are going to pose a significant challenge to the analyst, and it would be advantageous to reduce the magnitude of the dilution in preparation of our test solutions.

With regards to iron, the concentration is high enough that it could be readily measured by ICP-OES instead of by ICP-MS. Alternatively, the analyst may choose to dilute the media test solution further before measuring the iron concentration using ICP-MS. The analyst should also be aware that the ferric ammonium citrate contains additional transition elements (cobalt, chromium, copper, manganese, molybdenum, nickel, vanadium, and/or zinc), and the resulting experimental concentrations of these trace elements may be significantly higher than the anticipated concentrations stated in *Table 10*.

While the majority of the dissolved media composition is from glucose, the highest degree of analytical impact is going to come from the sodium and potassium ions.

The actual sodium and potassium concentrations from these two salts are as follows (see *Table 11*):

Table 11. Sodium/Potassium Concentrations in Example Media

Inorganic Salt	Concentration in Media (g/L)	Molecular Weight (g/mole)	Atomic Weight (g/mole)	Na/K Concentration in Media (g/L)	Na/K Concentration in Media (g/100 mL)
Sodium chloride	7	58.44	22.99	2.75	0.28
Potassium phosphate dibasic	7	174.2	39.098	3.14	0.31
Total	—	—	—	5.90	0.59

Note that this does not include the total compliment of sodium and potassium (and other counterions) contributed by other media component salts. For the purpose of this example, they are not included but in actual practice the concentrations of all salt counterions (such as sodium, potassium, magnesium, and calcium) should be taken into consideration.

By diluting the example media by a factor of 6, the total sodium/potassium concentration in the test solution becomes ~0.1 g/100 mL (0.1% or 1000 ppm). The trace element concentrations in the media test solutions diluted by a factor of 6 are presented in *Table 12* below:

Table 12. Trace Element Concentrations in 6-Fold Dilution of Example Media

Trace Element	Media Trace Element Concentration (µg/L)	Media Diluted 6-Fold Trace Element Concentration (µg/L)	Media Diluted 6-Fold Trace Element Concentration (ng/L)
Cobalt	0.107	0.0179	17.9

Trace Element	Media Trace Element Concentration (µg/L)	Media Diluted 6-Fold Trace Element Concentration (µg/L)	Media Diluted 6-Fold Trace Element Concentration (ng/L)
Chromium	0.00392	0.000653	0.653
Copper (chloride)	1.31	0.218	218
Copper (sulfate)	0.115	0.0192	19.2
Iron	131000	21900	—
Manganese	0.00494	0.000823	0.823
Molybdenum	0.0420	0.00700	7.00
Nickel	0.00266	0.000444	0.444
Selenium	0.717	0.119	119
Tin	0.00533	0.000888	0.888
Vanadium	0.0234	0.00390	3.90
Zinc	12.0	2.00	2005

The trace element concentrations presented in *Table 12* are much higher than those presented in *Table 10*. However, the impact of 1000 ppm sodium/potassium in the test solution has to be addressed, as does the ~2.8% of the other dissolved solids if the direct dissolution approach is to be applied to the media analysis.

First and foremost, the correct sample introduction equipment should be selected for the ICP-MS mass spectrometer, including the nebulizer, spray chamber, and torch injector, per manufacturer recommendations. The sampler and skimmer cones in the first vacuum interface region should also be selected based upon manufacturer recommendations for analysis of high-dissolved solids matrices.

The presence of the sodium/potassium ions in the mass spectrometer ion beam will dramatically impact the response of the elements relative to their mass. For our example media, the mass range is from vanadium (51 amu) to molybdenum (96 amu). The presence of sodium and potassium will likely enhance the vanadium response and suppress the molybdenum response relative to a working standard prepared in a dilute nitric acid matrix.

For a direct media dilution approach, there are two relatively straightforward remedies in this case. The first is to add sodium chloride to the working standard diluent to match the combined sodium/potassium concentration of the 6-fold diluted media test solutions, in this case, ~1000 ppm. Ultra-pure grade sodium chloride is commercially available that is for all practical purposes free from elemental contaminants. The second remedy is to use multiple internal standards that encompass the mass range of the analyte trace metals.

In the case that the ICP-MS mass spectrometer will not tolerate the high glucose concentration, the material can be digested to remove the carbonaceous media components. In that case, the counter-ions of the intrinsic inorganic salts will remain at the concentrations presented in *Table 6*. Given the low concentrations of the trace elements in the media, significant care needs to be taken to control any sources of contamination or loss of the target analytes.

The method of standard additions could also be applied to overcome the high solids sample matrix. However, the standard addition concentrations should be selected carefully to minimize the propagation of error around the concentration axis intercept via regression analysis and the accuracy verified through spiking or dilution experiments.

REFERENCES

1. International Council for Harmonisation (ICH). Q9: Quality Risk Management. 2005. *https://database.ich.org/sites/default/files/Q9%20Guideline.pdf*.
2. International Council for Harmonisation (ICH). Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities). 2012. *https://database.ich.org/sites/default/files/Q11%20Guideline.pdf*.
3. World Health Organization (WHO). Annex 4 WHO guidelines for sampling of pharmaceutical products and related materials. WHO Technical Report Series, No. 929. 2005. *https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/quality-control/trs929-annex4-guidelinessamplingpharmproducts.pdf*.
4. International Council for Harmonisation (ICH). Q7: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients. 2000.
5. Montaser A, Golightly DW. Inductively Coupled Plasmas in Analytical Atomic Spectrometry, 2nd Ed. NY: VCH Publishers, Inc.; 1992:614.
6. Larsen EH, Stürup S. Carbon-enhanced inductively coupled plasma mass spectrometric detection of arsenic and selenium and its application to arsenic speciation. *J. Anal. At. Spectrom*. 1994;9:1099–1105.
7. Reinhart D, Damjanovic L, Kaisermayer C, Kunert R. Benchmarking of commercially available CHO cell culture media for antibody production. *Appl Microbiol Biotechnol*. 2015;99(11):4645–4657.

8. Kenerson RW. Cell culture medium comprising transition metals or trace element. Table 1. Google Patent W02006004728A2, Application PCT/US2005/022889. 2005;13–22.

ADDITIONAL INFORMATION

- Brantley T, Moore B, Grinnell C, Khattak S. Investigating trace metal precipitation in highly concentrated cell culture media with Pourbaix diagrams. *Biotechnology and Bioengineering*. 2021;118(12):3888–3897.
- Graham RJ, Bhatia H, Yoon S. Consequences of trace metal variability and supplementation on Chinese hamster ovary (CHO) cell culture performance: A review of key mechanisms and considerations. *Biotechnology and Bioengineering*. 2019;116(12):3446–3456.
- Hossler P, Racicot C, McDermott S. Targeted shifting of protein glycosylation profiles in mammalian cell culture through media supplementation of cobalt. *J Glycobiology*. 2014;3(1).
- Selenius LA, Lundgren MW, Jawad R, Danielsson O, Björnstedt M. The cell culture medium affects growth, phenotype expression, and the response to selenium cytotoxicity in A549 and HepG2 cells. *Antioxidants*. 2019;8(4):130.
- Trunfio N, Lee H, Starkey J, Agarabi C, Liu J, Yoon S. Characterization of mammalian cell culture raw materials by combining spectroscopy and chemometrics. *Biotechnology Progress*. 2017;33(5):1127–1138.
- Zwolak I. Increased cytotoxicity of vanadium to CHO-K1 cells in the presence of inorganic selenium. *Bull Environ Contam Toxicol*. 2015;95(5):593–598.

▲ (USP 1-Aug-2024)

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<1023> EVALUATION STRATEGY FOR TRACE ELEMENTS IN CELL CULTURE MEDIA USED IN THE MANUFACTURE OF RECOMBINANT THERAPEUTIC PROTEINS	Rebecca C. Potts Associate Scientific Liaison	BIO2 Biologics Monographs 2 - Proteins

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 48(5)

Current DocID: GUID-D7036FA9-F30F-4AFD-8754-BF80BC3A6991_2_en-US
DOI: https://doi.org/10.31003/USPNF_M17255_02_01
DOI ref: ysber