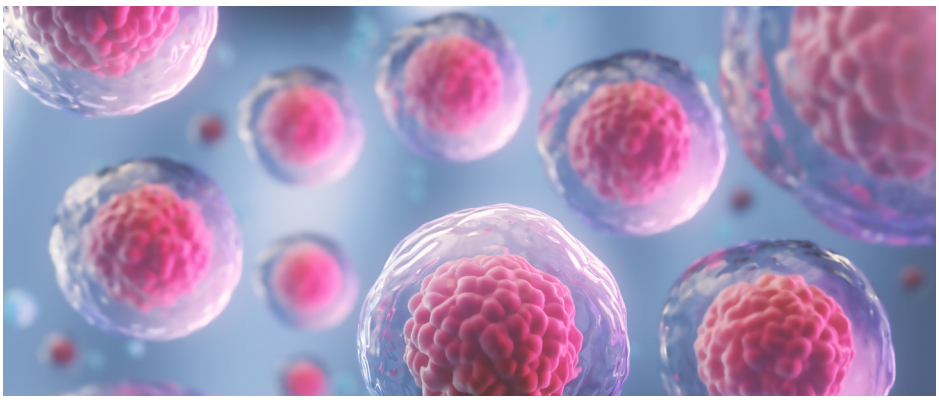
Application Note
Biopharma

Agilent
Trusted Answers

Elemental Analysis of Chemically Defined Cell Culture Media by ICP-MS

Multi-element method for development, optimization, production, and quality control of cell media using the Agilent 7900 ICP-MS



Authors

Yulan Bian and Aimei Zou

Agilent Technologies, Inc.

Introduction

Cell culture is a primary technology used in the manufacturing of biopharmaceuticals, vaccines, and other more complex methods of treatment (modalities) such as cell and stem therapy. Cells are cultivated in controlled, artificial environments (typically bioreactors) to produce the targeted products. Selecting appropriate growth media—natural or synthetic—is one of most important steps in this *in vitro* process. Typically, cell culture media is composed of amino acids, vitamins, inorganic salts, carbohydrates, lipids, and growth factors etc. Concerns over lot-to-lot variability of natural media, and microbial and viral contamination of natural media has led to the growing popularity of serum-free, chemically defined cell culture media (CDM). CDM is produced from the formulated concentrations of known media ingredients for each batch (*1*).

Inorganic salts are added to CDM to help maintain its pH and osmotic balance. More importantly, metal ions are essential enzyme cofactors and participate in cell signaling pathways, regulating biological processes of the cells. For example, zinc, copper, and manganese impact monoclonal antibody glycosylation (*2*) and zinc acts as a cell apoptosis suppressor (*3*). Therefore, the consistent composition of metal ions in CDM is crucial for product biopharmaceutical product yield, quality, and performance.

Despite using a predefined formulation, the elemental content of CDM can vary between production batches due to different sources of raw materials, leaching of metals from container materials, or contamination during manufacture. To ensure the quality and safety of drug products, regulatory bodies such as United State Pharmacopeia (USP) and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) publish guidelines. These guidelines specify maximum levels for contaminants, including elemental impurities, that a patient may be exposed to. However, there are currently no regulations, guidelines, or standard methods for the quality and safety control of elements in CDM. The need for an improved understanding of the level and impact of metals on production yields and product safety will likely increase as CDM is used in more complex biopharmaceutical modalities.

To identify and quantify elements in CDM, a multi-element method has been developed using the Agilent 7900 ICP-MS. Two serum-free growth media were selected, Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F-12 medium. Unlike the basal medium DMEM, Ham's F-12 is known to be fortified with iron, cobalt, and zinc. Comparative analysis was conducted between different lots, brands, and types of media. The work highlights the sensitivity, stability, and ease-of-use advantages of the 7900 ICP-MS for the analysis of CDM samples. The method is suitable for the development and optimization of the media composition and quality surveillance of CDM.

Experimental

Sample preparation

Three different lots of DMEM were bought from two manufacturers (referred to as manufacturer A and B). Another single lot of Ham's F-12 medium was obtained from manufacturer A. Trace metal–grade nitric acid (≥99.999% purity) was bought from Sigma Aldrich. Ultrahigh purity water (UPW) with a resistivity of 18.2 MΩ produced using a Milli-Q water purification system was used for sample and standard preparation.

Samples were prepared by 10-fold direct dilution with 2% HNO_3. A 5 g aliquot of each sample was added to a 50 mL falcon tube and diluted in 2% HNO_3 to 50 g.

Instrumentation

A 7900 ICP-MS that includes the ORS^4 collision/reaction cell (CRC) for was used for the analysis. The ORS^4 was operated in helium (He) mode, which removes typical polyatomic ion interferences on all common analyte ions using kinetic energy discrimination (KED). KED selectively filters out the larger, lower energy polyatomic ions, allowing the faster analyte ions to be detected, greatly improving the accuracy of the measurements (*4*).

The standard ICP-MS sample introduction system was used, comprising nickel sampling and skimmer cones, a MicroMist glass concentric nebulizer, quartz spray chamber chilled at 2 °C, and quartz torch with 2.5 mm injector. An 89-rack Agilent I-AS autosampler was used to introduce the samples to the 7900 ICP-MS. A preset method for General Purpose applications was selected from the Agilent ICP-MS MassHunter software. The preset method automatically sets the plasma conditions and other analytical parameters that are required for the application, simplifying method development. The parameters in the shaded rows in Table 1 were set automatically by the software and the lens voltages were autotuned once only for all elements.

Table 1. Agilent 7900 ICP-MS operating conditions. The shaded parameters were defined automatically by selecting the General Purpose preset method in ICP-MS MassHunter.

Parameters	Settings
Cell Mode	Helium
RF power (W)	1550
Spray Chamber Temp (°C)	2
Sampling Depth (mm)	10
Carrier Gas Flow (L/min)	1.08
Extract 1 (V)	0.0
Extract 2 (V)	–170.0
Omega Bias (V)	–90
Omega Lens (V)	10.6
Deflect (V)	2.0
He Gas Flow (mL/min)	5.0
KED (V)	5.0

Results and discussion

Calibration, quality control, and internal standards

Agilent multi-element standard (p/n 5183-4688) and single strontium standard (p/n 5190-8581) were used to prepare the intermediate working standard and calibration standards. The internal standard (ISTD) solution was prepared from an Agilent internal standard stock solution for ICP-MS (p/n 5188-6525). The ISTD was added to the sample using the standard online ISTD kit.

Calibration standards were prepared in 2% HNO_3 to match the sample solutions. The calibration range for major elements was 0, 0.005, 0.01, 0.05, 0.25, 1, 5, 20, 40 ppm. And the range for trace elements was 0, 0.05, 0.1, 0.5, 2.5, 10, 50, 200, 400 ppb. Quality control (QC) standards for major and trace elements were prepared at 10 ppm and 100 ppb, respectively. The ISTD standard was prepared at 100 ppb in 2% HNO_3. Representative calibration curves for a major and trace element are shown in Figure 1.

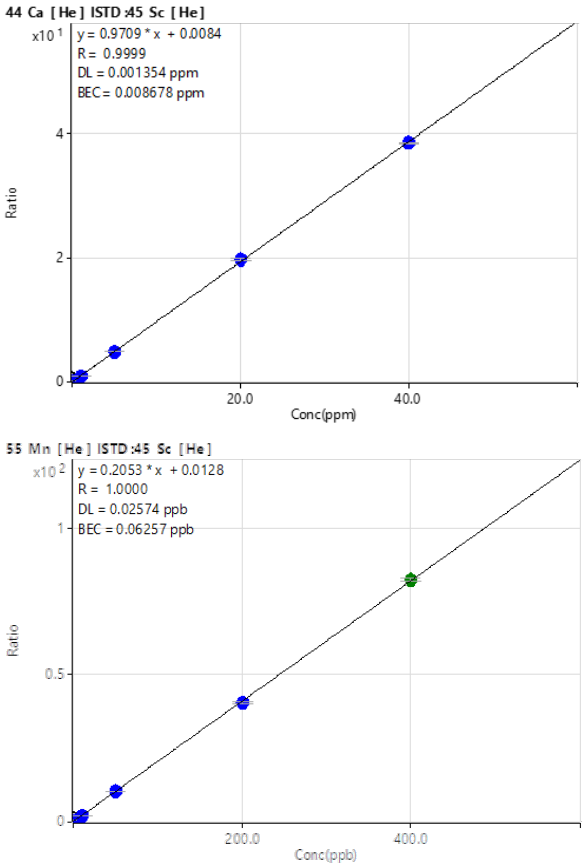


Figure 1. Representative calibration curves for a major element, Ca, and a trace element, Mn.

Analytical sequence

First, the calibration standards were analyzed, followed by a series of calibration blanks and an Initial Calibration Verification (ICV) solution. This sample block was repeated, followed by the analysis of the spiked sample block. After every 10 samples, a Continuing Calibration Verification (CCV) sample was analyzed.

Typical 7900 ICP-MS instrument detection limits (DLs) calculated from the ICP-MS MassHunter calibrations are shown in Table 2. The calibration coefficients show that good linearity (>0.999) was achieved for all elements across the calibrated range. The wide dynamic range of the 7900 allows measurement of major and trace elements in one run.

Table 2. Detection limits and calibration coefficients.

Element	Isotope	Units	DL	R
Mg	24	ppm	0.0002	0.9998
K	39	ppm	0.0023	0.9999
Ca	44	ppm	0.0013	0.9999
Fe	56	ppm	0.0001	0.9998
Al	27	ppb	0.3416	1.0000
V	51	ppb	0.0013	0.9998
Cr	52	ppb	0.0804	0.9997
Mn	55	ppb	0.0257	1.0000
Co	59	ppb	0.0028	0.9998
Ni	60	ppb	0.1056	0.9999
Cu	63	ppb	0.0028	0.9998
Zn	66	ppb	0.0593	0.9999
As	75	ppb	0.0036	0.9999
Se	78	ppb	0.0969	1.0000
Sr	88	ppb	0.0096	0.9998
Cd	114	ppb	0.0004	0.9999
Sb	121	ppb	0.0021	1.0000
Ba	137	ppb	0.0065	1.0000
Tl	205	ppb	0.0003	1.0000
*Pb	208	ppb	0.0005	1.0000

**Pb is normally reported from the sum of the three most abundant isotopes, 206, 207, and 208.*

Spike recoveries

Since there are no available certified reference materials available for CDM, the accuracy of the method was evaluated by spiking the sample matrices and measuring the recoveries. A DMEM sample from manufacturer A (lot 2) and a Ham's F-12 sample were spiked with trace elements at 5 ppb and major elements at 5 ppm. The average spike recovery results for all elements in the fortified cell media samples ranged from 95 to 115% (Table 3). The excellent spike recovery data demonstrates the accuracy of the 7900 ICP-MS quantitative method for the analysis of major and trace elements in CDM.

Table 3. Average spike recovery results of DMEM A2 and Ham's F-12, n=2.

			DMEM A2			Ham's F-12		
Element	Units	Spike Level	Measured Conc in Unspiked Sample	Measured Conc in Spiked Sample	Recovery (%)	Measured Conc in Unspiked Sample	Measured Conc in Spiked Sample	Recovery (%)
Mg	ppm	5	1.97	7.10	103	1.42	6.57	103
K	ppm	5	22.30	27.90	112	12.68	17.85	103
Ca	ppm	5	6.77	12.27	110	1.21	6.57	107
Fe	ppm	5	<DL	5.29	106	0.01	5.28	105
Al	ppb	5	<DL	5.52	110	<DL	5.06	101
V	ppb	5	0.04	5.25	104	0.01	5.14	103
Cr	ppb	5	<DL	5.15	103	<DL	5.13	103
Mn	ppb	5	0.19	5.31	102	<DL	5.17	103
Co	ppb	5	<DL	5.27	105	4.18	8.91	95
Ni	ppb	5	<DL	4.87	97	<DL	4.86	97
Cu	ppb	5	<DL	5.26	105	0.07	5.32	105
Zn	ppb	5	<DL	5.58	112	19.58	25.33	115
As	ppb	5	<DL	5.60	112	<DL	5.47	109
Se	ppb	5	<DL	5.72	114	<DL	5.48	110
Sr	ppb	5	1.94	6.97	101	0.37	5.49	102
Cd	ppb	5	<DL	4.99	100	<DL	4.98	100
Sb	ppb	5	0.03	5.20	103	0.02	5.15	103
Ba	ppb	5	0.29	5.57	106	0.08	5.37	106
Tl	ppb	5	<DL	5.44	109	<DL	5.42	108
Pb	ppb	5	0.02	5.40	108	<DL	5.40	108

Long-term stability tests

Figure 2 shows the stability of the ISTD signal throughout the eight-hour analytical sequence. The ISTD recoveries for all samples were within 80–120% of the value in the calibration blank standard. The midpoint of the calibration standards was used as the CCV standard.

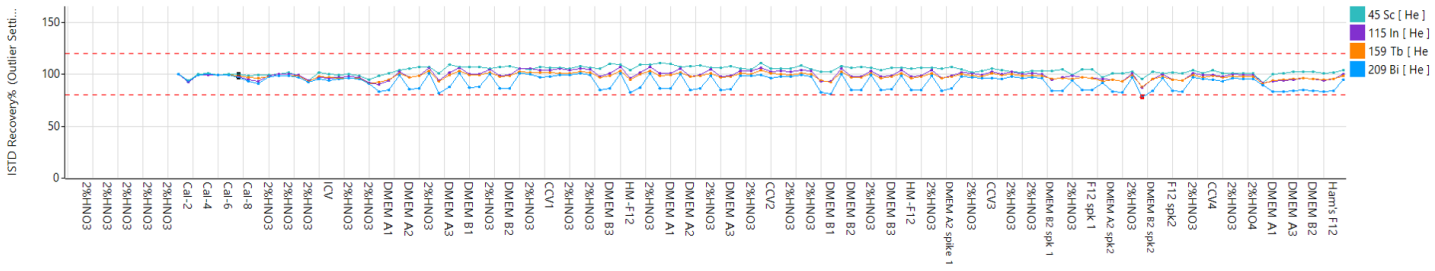


Figure 2. ISTD signal stability measured by the Agilent 7900 ICP-MS over eight hours. Red dotted lines indicate ±20%.

The recovery of the CCV over eight hours was within ±10% for all elements, as shown in Figure 3. Both sets of recovery results demonstrate the long-term robustness and high matrix tolerance of the 7900 ICP-MS and its suitability for the analysis of cell culture media samples over long runs.

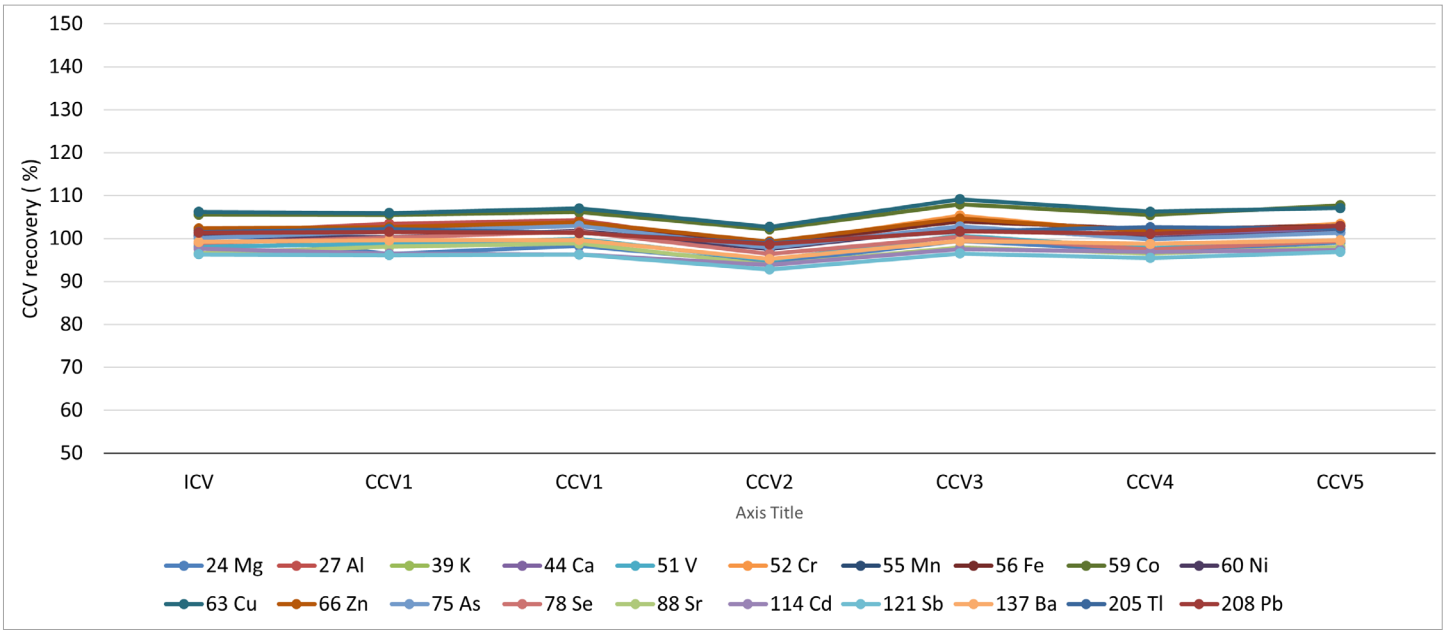


Figure 3. CCV recovery for all elements over the eight-hour analysis.

Quantitative sample analysis results

Quantitative results (corrected for the dilution factor) for all elements in DMEM and Ham's F-12 samples are given in Table 4. The DL is reported for any elements that were not detected.

Table 4. Mean quantitative results for three lots of two brands of DMEM and for Ham's F-12, n=4.

		DMEM						HAM's F-12
		Brand A			Brand B			
Element	Units	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	
Mg	ppm	19.33 ± 0.72	19.75 ± 0.13	20.00 ± 0.76	17.3 ± 0.41	17.79 ± 0.09	18.58 ± 0.30	14.23 ± 0.31
K	ppm	220.18 ± 2.62	223.00 ± 1.25	222.98 ± 1.25	223.31 ± 5.38	218.29 ± 1.15	220.56 ± 2.46	126.88 ± 3.23
Ca	ppm	70.25 ± 0.99	67.70 ± 0.30	68.35 ± 2.61	65.40 ± 1.25	66.47 ± 0.26	69.27 ± 0.65	12.06 ± 0.29
Fe	ppm	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.12 ± 0.003
Al	ppb	<3.41	<3.41	<3.41	<3.41	<3.41	<3.41	<3.41
V	ppb	0.20 ± 0.01	0.35±0.01	0.34 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	0.13 ± 0.01
Cr	ppb	<0.80	<0.80	<0.80	<0.80	<0.80	<0.80	<0.80
Mn	ppb	<0.25	1.93 ± 0.08	3.36 ± 0.17	1.64 ± 0.09	1.98 ± 0.09	3.16 ± 0.07	<0.25
Co	ppb	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	41.81 ± 4.23
Ni	ppb	<1.05	<1.05	<1.05	<1.05	<1.05	<1.05	<1.05
Cu	ppb	<0.03	<0.03	<0.30	0.76 ± 0.09	<0.030	<0.030	0.74 ± 0.001
Zn	ppb	<0.59	<0.59	<0.59	21.64 ± 1.01	21.07 ± 0.33	23.28 ± 0.54	195.83 ± 1.74
As	ppb	0.20 ± 0.03	0.23 ± 0.02	0.25 ± 0.01	<0.03	<0.03	<0.03	<0.03
Se	ppb	<0.96	<0.96	<0.96	<0.96	<0.96	<0.96	<0.96
Sr	ppb	30.97 ± 0.69	19.36 ± 0.26	21.01 ± 0.86	21.85 ± 0.8	21.62 ± 0.24	23.61 ± 0.22	3.69 ± 0.06
Cd	ppb	<0.004	<0.004	<0.004	0.05 ± 0.002	0.05 ± 0.002	0.04 ± 0.004	<0.004
Sb	ppb	0.21 ± 0.01	0.29 ± 0.02	0.33 ± 0.01	0.21 ± 0.02	0.20 ± 0.02	0.26 ± 0.02	0.21 ± 0.01
Ba	ppb	3.02 ± 0.05	2.93 ± 0.07	3.07 ± 0.05	0.91 ± 0.06	0.89 ± 0.03	0.57 ± 0.08	0.76 ± 0.05
Tl	ppb	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Pb	ppb	0.10 ± 0.03	0.16 ± 0.008	0.22 ± 0.005	0.20 ± 0.005	0.28 ± 0.005	0.18 ± 0.008	<0.005

Major elements in cell media

The results in Table 4 show that the concentration profile of the major elements in the two brands of DMEM is similar. From the lot-to-lot results, the concentrations of the major elements in DMEM were consistent, with a variation of <4% among the lots from the same brand. Brand A seems to have slightly better lot-to-lot consistency than brand B, as shown in Figure 4. Major elements are added to the media in the form of buffer salts to maintain its pH and osmolality. Keeping media pH and osmolality consistent between lots is fundamental to creating a stable cell-culture environment for cells to grow.

When comparing different types of media, a clear distinction can be observed between the major element profile of DMEM and Ham's F-12. Ham's F-12 is fortified with Fe, as shown in the data, while no Fe was detected in DMEM. The concentrations of K and Ca were much lower in Ham's F-12 than in DMEM. The difference could be attributed to the specific use of each media. DMEM is a basal medium for generic use, whereas Ham's F-12 was originally developed for use in cloning Chinese hamster ovary and lung cells, which might benefit from a less ionic environment.

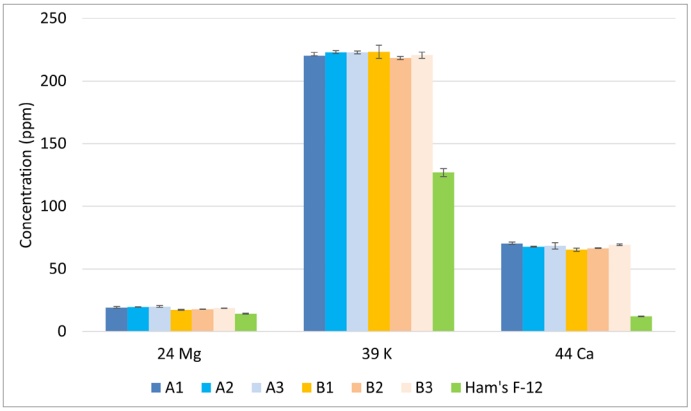


Figure 4. Comparison of Mg, K, and Ca in all cell media samples. A1, A2, and A3 represent lots of DMEM from manufacturer A. B1, B2, B2 represent lots of DMEM from manufacturer B.

Trace elements in cell media

The most striking difference between the two brands of DMEM is the presence of Zn in brand B but not in brand A, as shown in Figure 5. According to the literature, Zn is involved in critical cell biochemical processes (*3*). The concentration of Zn in the brand B DMEM sample suggests it was fortified to enhance the performance of the media. Sr, which supports stem cell production (*5*), was detected in all DMEM samples at a similar level.

There was a distinct difference in the concentrations of Co, Zn, and Sr in the two types of cell media (Figure 5). The relatively high levels of Co and Zn in Ham's F-12 were expected, as these elements are used as inorganic nutrients to enhance growth of CHO cells and other cancer cells. Sr was also detected in Ham's F-12, but at a lower concentration compared to DMEM.

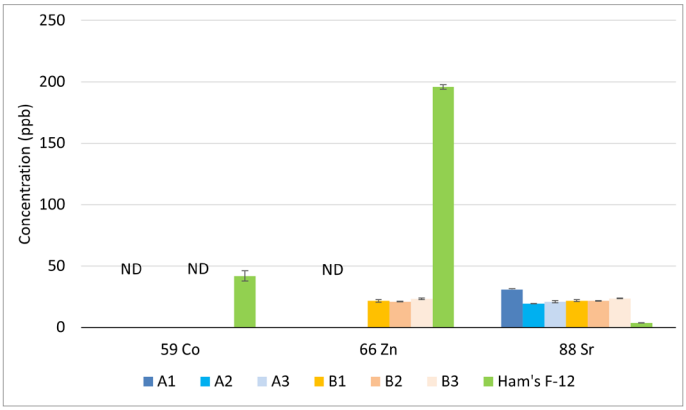


Figure 5. Comparison of Co, Zn, and Sr in DMEM and Ham's F-12 cell media samples. ND=not detected, concentration below DL.

Other elements such as V, Mn, As, Cd, Sb, Ba, and Pb, which were detected at low- or sub-ppb levels, could be impurities introduced from the raw materials used to prepare the media. There was some consistency in the lot-to-lot comparisons of the concentrations of these elements in DMEM. For example, As and Ba were detected at around 0.2 and 3.0 ppb respectively across the three lots of DMEM brand A. If sufficient data was collected from a greater number of lots, a reference trace element profile could be constructed with statistical confidence to serve as a benchmark for the QC of cell media.

Semiquantitative data using IntelliQuant

Preset methods in ICP-MS MassHunter automatically acquire full mass-spectrum data for a sample using IntelliQuant Quick Scan acquisition in He mode (*6*), unless the scan is unselected. No special setup or separate calibration is needed for IntelliQuant, simplifying the analysis. IntelliQuant automatically acquires full mass-spectrum data in every sample with only two seconds extra measurement time, allowing the analyst to quickly see which elements are present in the samples. Because IntelliQuant data is acquired in He mode, analytes are free from common polyatomic ion overlaps, ensuring the quality of the data.

In this study, IntelliQuant data was acquired for each cell media sample. The data can be displayed in a periodic table heat map view, as shown in Figure 6. The color intensity of the element represents the concentration measured, with a darker color indicating a higher concentration. Elements such as Na, K, P, S, and Cl were not included in the quantitative method, but they were identified and semi-quantified by IntelliQuant in DMEM. Analysts can use IntelliQuant data to quickly see any sample-to-sample variation and identify the presence of any unexpected elements in a sample. A higher level of Fe, Co, Cu, and Zn was observed in HAM's F-12 compared to DMEM B3 (Figure 6), which was in line with the full quantitative results (Table 4).

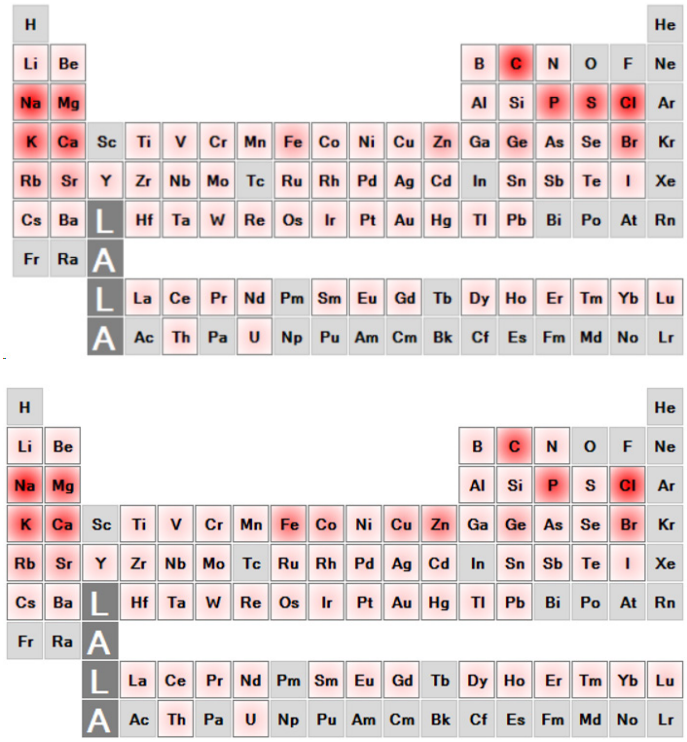


Figure 6. Periodic table heat map view of ICP-MS IntelliQuant data acquired for DMEM B3 sample (top) and Ham's F-12 (bottom).

Conclusion

The study highlighted the excellent robustness, sensitivity, and stability of the Agilent 7900 ICP-MS for the analysis of trace and major elements in chemically defined cell culture media (CDM). A predefined, General Purpose preset method was used to simplify method development for the analysis of DMEM and Ham's F-12, while autotuning was used to ensure reproducible performance regardless of operator experience.

All data was acquired by the 7900 with the ORS^4 in He-KED mode, which effectively removed typical polyatomic interferences on all analytes. The accuracy of the quantitative method was demonstrated by the spike recoveries between 95 and 115% for all elements in the fortified cell media samples. IntelliQuant semiquantitative data was also acquired in He-KED mode as part of the method, providing a visual "periodic table" overview of all elements present in the samples and their concentration levels. The stability of the ISTD and CCV measurements over eight-hours was due to the robustness of the ICP-MS plasma and high matrix tolerance of the instrument. Instrument stability is important for a lab's productivity, as it reduces QC failures and need for sample reanalysis.

Having a full understanding of the elemental composition of CDM will help media manufacturers optimize the composition of cell-growth media, as well as quality assure existing products. High quality and consistent formulations of CDM are important factors that influence the safety, yield, and effectiveness of final biotherapeutic products.

References

1. Tatsuma Yao, Yuta Asayama, Animal-cell culture media: History, characteristics, and current issues. *Reproductive Medicine and Biology*, 16(2), **2017**, 99–117
2. Melissa M. St. Amand *et al.* Identification of manipulated variables for a glycosylation control strategy. *Biotechnology and Bioengineering*, 111(10), **2014**, 1957–1970
3. Ryan J. Graham *et al.* Zinc supplementation improves the harvest purity of β-glucuronidase from CHO cell culture by suppressing apoptosis. *Applied Microbiology and Biotechnology*, 104, **2020**,1097–1108
4. Octopole Collision/Reaction Cell and Helium mode, Agilent publication, 5994-1172EN
5. V. Nardone *et al.* In Vitro Effects of Strontium on Proliferation and Osteoinduction of Human Preadipocytes. *Stem Cells International* (Special Issue), **2015**, 12
6. Agilent ICP-MS IntelliQuant Software, Agilent publication, 5994-1677EN

www.agilent.com/chem/7900icp-ms

DE68217309

Printed in the USA, October 25, 2022
5994-5355EN