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Multi-Elemental Analysis of Chemically Defined Cell Culture Media Using ICP-MS

Yulan Bian[1], Aimei Zou[1] and Patrick Simmons[2]

[1]Agilent Technologies, Inc., Singapore

[2]Agilent Technologies, Inc., Cedar Creek, Texas

Introduction

Cell culture is a primary technology used in the manufacturing of biopharmaceuticals, vaccines, and other more complex modalities. Cell culture media is composed of amino acids, vitamins, inorganic salts, sugar, 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) (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). The consistent composition of metal ions in CDM is crucial for biopharmaceutical product yield, quality, and performance.

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. The work highlights the sensitivity, stability, wide dynamic range and ease-of-use of the 7900 ICP-MS.

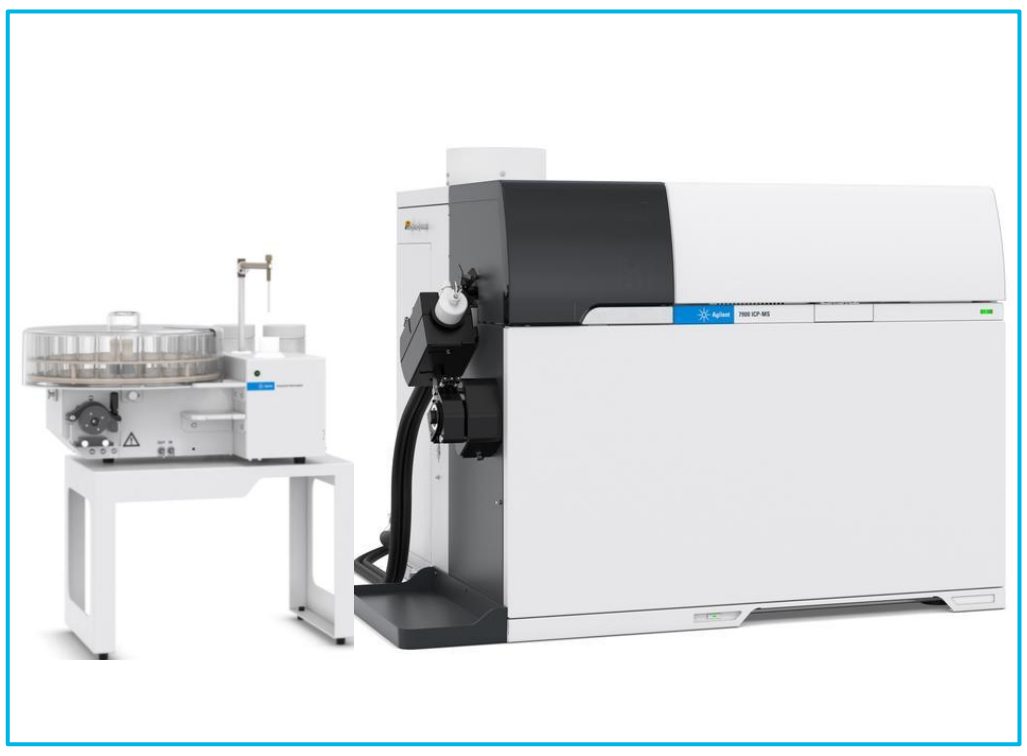

Figure 1. Agilent 7900 ICP-MS coupled with I-AS autosampler

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 ORS4 collision/reaction cell (CRC) was used for the analysis. The ORS4 was operated in helium (He) mode, which removes typical polyatomic ion interferences on all common analyte ions using kinetic energy discrimination (KED).

The standard ICP-MS sample introduction system was used. 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 lens voltages were autotuned once only for all elements. The parameters are shown in Table 1.

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

Table 1. Agilent 7900 ICP-MS operating conditions.

Calibration and Stability

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. All the calibrants were prepared in 2% HNO_3.

Linearity of all elements was >0.999 and QC recoveries were within 90-100% for all elements over 8-hour run (Figure 2).

The internal standard (ISTD) was prepared from an Agilent internal standard stock solution and was added to the sample using the standard online ISTD kit. Excellent stability of ISTD was demonstrated in Figure 3.

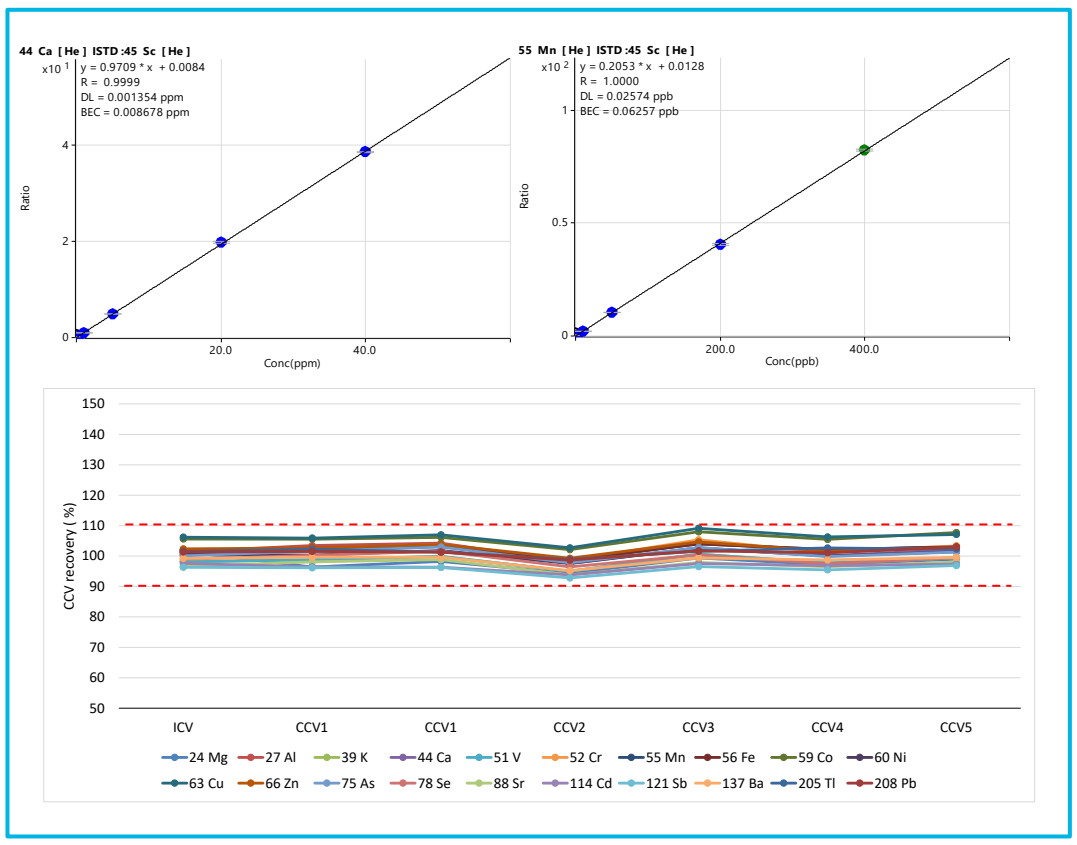

Figure 2. Linearity and long-term stability. Top: representative calibration curves of Ca(44) and Mn(55) Bottom: Recoveries of elements in QC against blank across 8-hour run

			DMEM A2			Ham's F-12		
Element	Isotope	Spike Level	Measured Conc. in Unspiked	Measured Conc. in Spiked	Recovery (%)	Measured Conc. in Unspiked	Measured Conc. in Spiked	Recovery (%)
Mg	24	5ppm	1.97	7.10	103	1.42	6.57	103
K	39	5ppm	22.30	27.90	112	12.68	17.85	103
Ca	44	5ppm	6.77	12.27	110	1.21	6.57	107
Fe	56	5ppm	<0.001	5.29	106	0.01	5.28	105
Al	27	5ppb	<0.34	5.52	110	<0.34	5.06	101
V	51	5ppb	0.04	5.25	104	0.01	5.14	103
Cr	52	5ppb	<0.08	5.15	103	<0.08	5.13	103
Mn	55	5ppb	0.19	5.31	102	<0.02	5.17	103
Co	59	5ppb	<0.002	5.27	105	4.18	8.91	95
Ni	60	5ppb	<0.11	4.87	97	<0.11	4.86	97
Cu	63	5ppb	<0.002	5.26	105	0.07	5.32	105
Zn	66	5ppb	<0.05	5.58	112	19.58	25.33	115
As	75	5ppb	<0.003	5.60	112	<0.003	5.47	109
Se	78	5ppb	<0.09	5.72	114	<0.09	5.48	110
Sr	88	5ppb	1.94	6.97	101	0.37	5.49	102
Cd	114	5ppb	<0.0004	4.99	100	<0.0004	4.98	100
Sb	121	5ppb	0.03	5.20	103	0.02	5.15	103
Ba	137	5ppb	0.29	5.57	106	0.08	5.37	106
Tl	205	5ppb	<0.0003	5.44	109	<0.0003	5.42	108
Pb	208	5ppb	0.02	5.40	108	<0.0005	5.40	108

Table 2. Spike recovery results of 5 ppm and 5 ppb for major and trace metals respectively in DMEM A2 and Ham's F-12 samples.

Accuracy

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 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 ranged from 95 to 115% as shown in Table 2. The excellent spike recovery data demonstrates the accuracy and wide dynamic range of the 7900 ICP-MS quantitative method for the analysis of major and trace elements in CDM.

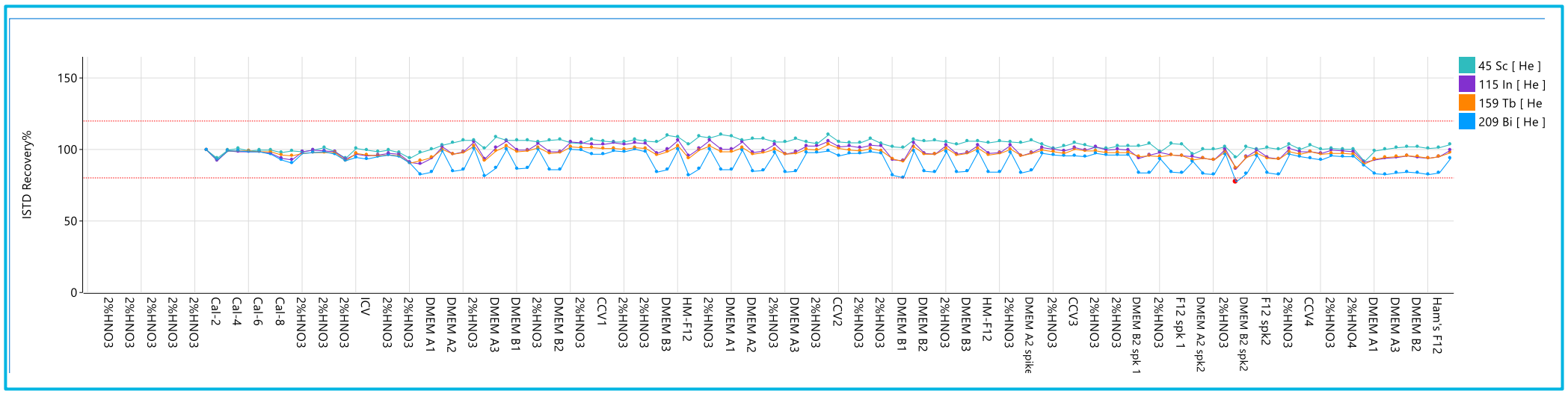

Figure 3. ISTD signal stability measured over eight hours. Red dotted lines indicate +20% variation.

Results and Discussion

Major Metals

From the lot-to-lot results, variation of <4% was observed 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.

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 while no Fe was detected in DMEM. The concentrations of K and Ca were much lower in Ham's F-12 than in DMEM.

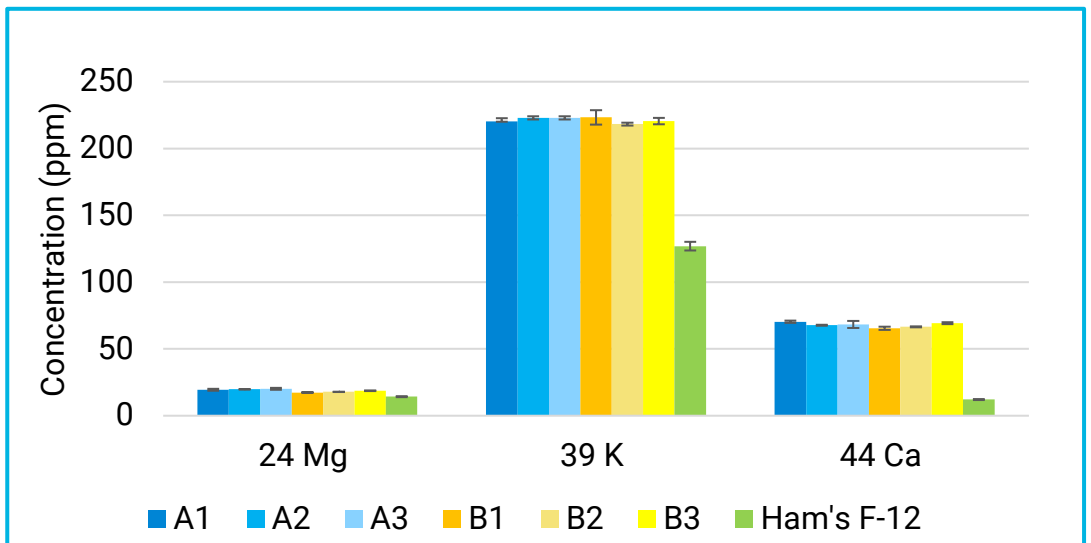

Figure 4. Comparison of Mg, K, and Ca in CDM samples.

Trace Metals

The 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.

There was also 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 to enhance growth of CHO cells as Zn is reported to be involved in critical cell biochemical processes (3).

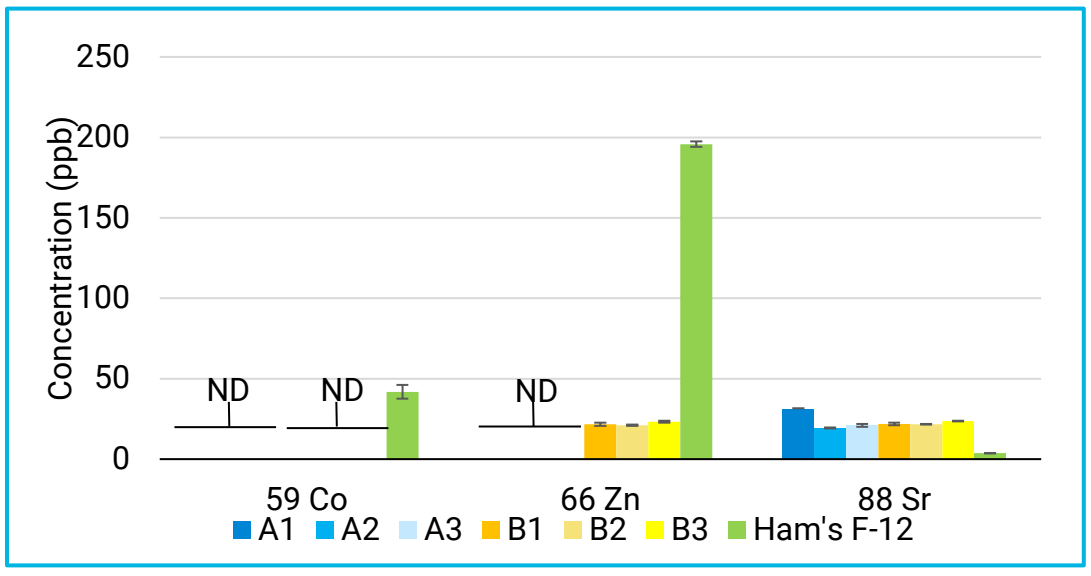

Figure 5. Comparison of Co, Zn, and Sr in DMEM and Ham's F-12 CDM samples.

Conclusions

The study highlighted the excellent sensitivity, stability, dynamic range and ease-of use 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, while autotuning was used to ensure reproducible performance regardless of operator experience.
- The wide dynamic range of the instrument enables the detection of both major and trace metals in one single run.
- The stability of the ISTD and CCV measurements over eight hours showcased the robustness of the ICP-MS plasma and high matrix tolerance of the instrument.
- The accuracy of the quantitative method was demonstrated by good spike recoveries for all elements in the fortified cell media samples.

Having a full understanding of the elemental composition of CDM will help media manufacturers deliver high quality and consistent CDM products, which impact the yield, and effectiveness of final biotherapeutic products.

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