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Application News

Inductively Coupled Plasma Mass Spectrometer ICPMS-2030

Monitoring of Metal Elements in CHO Cell Culture Supernatant Using ICPMS-2030

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User Benefits

- Simultaneous analysis is possible for metal elements in CHO cell culture supernatant.
- The cell culture supernatant can be analyzed by using the ICPMS-2030 with only dilution.

■ Introduction

The active pharmaceutical ingredients (API) of antibody drugs are mainly produced by culturing CHO (Chinese hamster ovary) cells. In recent years, it has been reported that the metabolism of cultured cells and the quality of antibodies produced are affected by changes in the concentration of metal elements in the culture medium. For example, it is known that the lactate metabolism of CHO cells changes according to the concentration of Cu in the culture medium,[1] and the glycosylation of antibodies changes according to the Mn/Zn ratio in the culture medium.[2] Therefore, in order to maintain the quality of antibody drugs, it is important to simultaneously monitor changes over time in the concentrations of multiple metal elements in the medium and culture supernatant.

The measurement examples of the medium and culture supernatant by AAS were shown in the Application News A634[3] and A651[4]. On the other hand, ICP-MS is considered suitable for trace elements analysis and simultaneous monitoring of multiple elements . The Application News 01-00372[5] showed that the spike recovery rate and the long-term stability of culture medium measurements confirmed that the ICPMS-2030 is useful for medium measurements. In this report, we introduce an example of monitoring metal elements with the ICPMS-2030 by diluting the culture medium/culture supernatant of CHO cells.

■ Samples

It is known that CHO cells have variations in cell proliferation and productivity of antibodies even after cloning due to differences in chromosome numbers and the location of transgenes.

Here, two different cell lines were obtained by subcloning the clone that was obtained by limiting dilution, and the culture supernatant was recovered every other day (Fig. 1). A common medium was used for the culture, and the fresh medium was designated Day 0.

The collected medium and culture supernatant were diluted 20 times with 1 v/v% nitric acid and used as analytical samples.

■ Measuring Elements

Nine elements (Co, Cu, Fe, Mg, Mn, Mo, Ni, Se, and Zn) were selected as the elements to be measured, as they are reported to affect cell growth and the quality of their products in CHO cells.

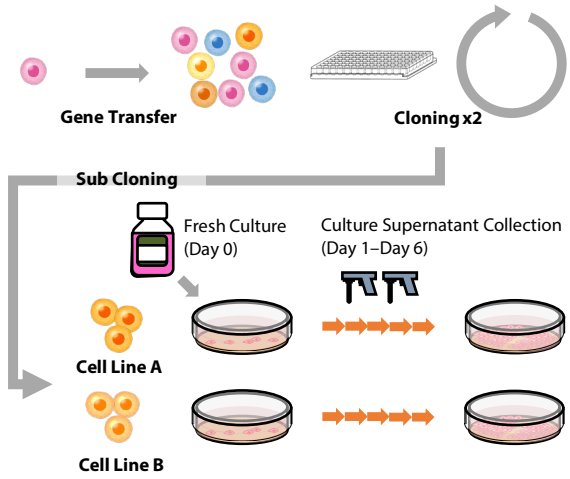

Fig. 1 Overview of Sample Preparation

■ Calibration Solutions

Calibration solutions were prepared by mixing commercially available single-element standard solutions and diluting with 1 v/v% nitric acid. The concentrations contained in each calibration solutions are shown in Table 1.

Table 1 Concentration of Elements in Calibration Solutions

Elements	Calibration Solutions (µg/L)			
	STD 0	STD 1	STD 2	STD 3
Co	0	10	50	100
Cu	0	0.5	2.5	5
Fe	0	100	500	1000
Mg	0	500	2500	5000
Mn	0	0.2	1	2
Mo	0	0.1	0.5	1
Ni	0	0.1	0.5	1
Se	0	0.2	1	2
Zn	0	20	100	200

■ Internal Standard Elements

Ga, In, Sc, and Y were added as internal standard elements in the Internal Standard Automatic Addition Kit.

■ Analytical Conditions

The instrument configuration and analytical conditions are summarized in Table 2.

Table 2 Conditions and Parameters of ICPMS-2030

Instrument	:	ICPMS-2030
RF Power	:	1.2 kW
Plasma Gas	:	9.0 L/min
Auxiliary Gas	:	1.1 L/min
Carrier Gas	:	0.7 L/min
Nebulizer	:	Nebulizer, 07 UES
Pump Speed	:	20 r.p.m.
Chamber	:	Cyclone Chamber
Torch	:	Mini-Torch
Sampling Cone/ Skimmer Cone	:	Copper
Cell Gas	:	He

■ Spike Recovery Test

Cell line A from the early (Day 1), middle (Day 3), and late (Day 6) phases in culture was used for the spike recovery test to check for matrix interference. The results and the limit of quantitation (LOQ) are shown in Table 3. Measured data was converted to the concentration of each element in the culture supernatant. The spike recovery rates for all samples were within ± 20 %, and it was confirmed that low interference was observed. The measured data was well above the LOQ, which proved that the ICPMS-2030 is sensitive enough to monitor the metal elements in the medium and culture supernatant.

Table 3 Spike Recovery Test Results

Unit: µg/L

Sample	Element	Co	Cu	Fe	Mg	Mn	Mo	Ni	Se	Zn
	LOQ	2	2	0.8	10	0.6	0.2	0.6	0.8	1
	Spike Conc.	200	10	2000	10000	4	2	2	4	400
D1	Unspiked Sample	366	13.6	2780	14600	5.2	1.22	1.0	5.4	880
	Spiked Sample	568	24.0	4760	24400	9.2	3.18	3.0	10.2	1280
	Recovery (%)	**101**	**104**	**99**	**98**	**100**	**98**	**100**	**120**	**100**
D3	Unspiked Sample	380	14.6	2880	15000	8.2	4.68	1.0	5.6	910
	Spiked Sample	578	25.0	4860	25000	11.8	6.86	3.2	10.0	1300
	Recovery (%)	**99**	**104**	**99**	**100**	**90**	**109**	**110**	**110**	**98**
D6	Unspiked Sample	430	13.6	3140	16300	6.2	0.90	1.2	4.8	950
	Spiked Sample	618	23.0	5060	26000	10.2	2.96	3.2	8.8	1320
	Recovery (%)	**94**	**94**	**96**	**97**	**100**	**103**	**100**	**100**	**93**

- LOQ (Converted to medium and culture supernatant) = 10σ × dilution rate (σ: standard deviation of calibration solution's blank)
- Recovery (%) = (Spiked sample – unspiked sample)/Spike Conc. × 100

■ Results

The results of the six- day monitoring of the concentrations of each element in the medium and culture supernatant are shown in Fig. 2.
There were differences of more than 1.5 times in the concentrations of Mn, Mo, and Ni in the specific period, while there were almost no differences in the concentrations of Co, Cu, Fe, Mg, Se, and Zn among the cell lines.

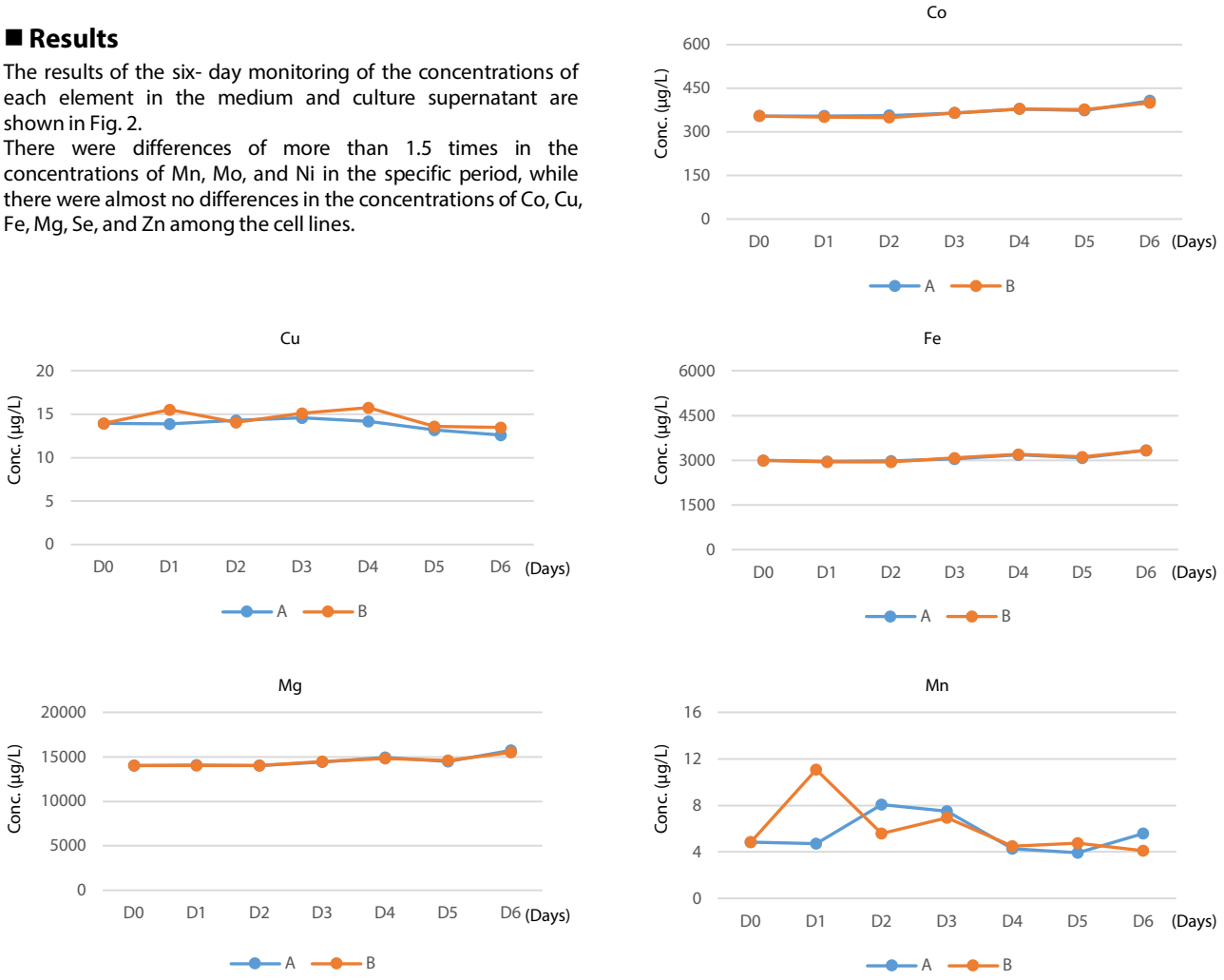

Fig. 2 Results of Culture Supernatant (Converted to Concentration in Medium and Culture Supernatant)

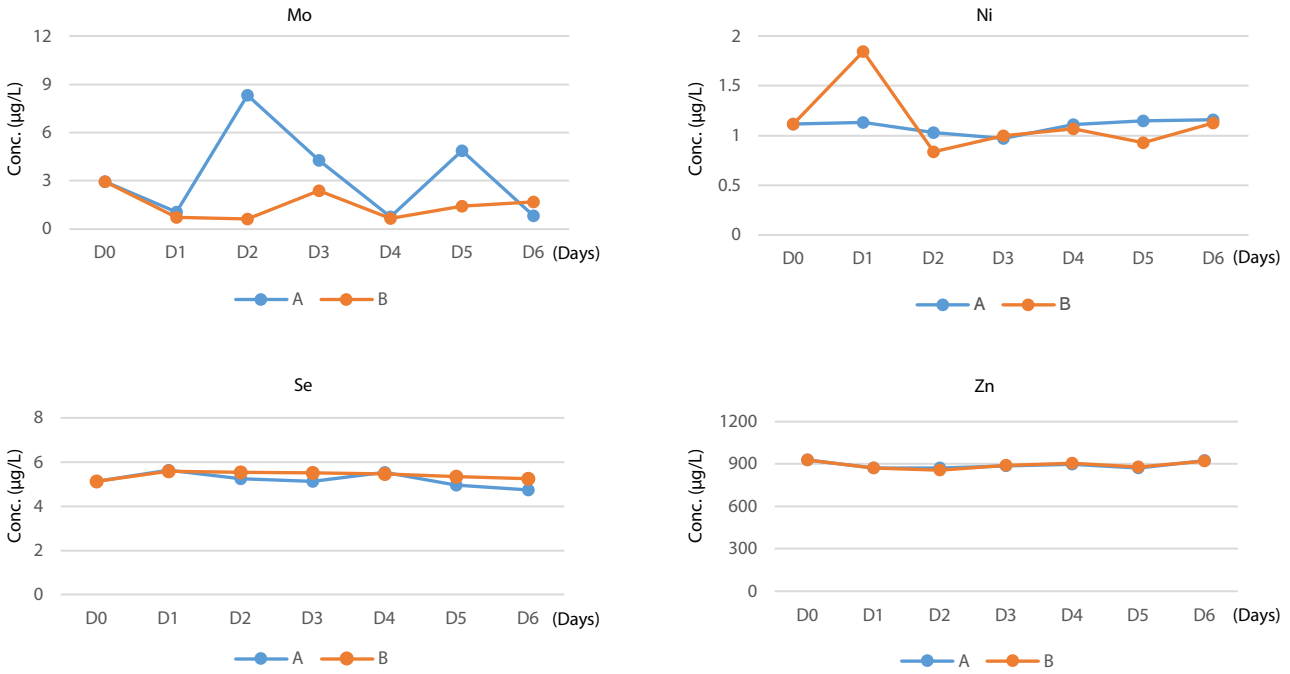

Fig. 2 Results of CHO Culture Supernatant (Converted to Concentration in Medium and Culture Supernatant) (Continued)

■ Conclusion

The culture medium and culture supernatants of CHO cells were measured by the ICPMS-2030 with only dilution. We report on an example of monitoring mainly metal elements that are reported to affect cell proliferation and antibody production. The monitoring of multiple elements, as shown in the present results, is expected to help identify characteristic indicators in the conditions.

■ Related Product

Shimadzu Corporation also provides analytical solutions for organic components, such as amino acids, nucleic acids, vitamins, and secretory metabolites, in medium and culture supernatants. The LC/MS/MS Method Package Cell Culture Profiling Ver. 2 can within 20 minutes perform simultaneous analysis of up to 125 components consisting of medium components and secretory metabolites from cells.

The Application News C209 shows an example of analysis of culture medium/culture supernatant using this method.

LC/MS/MS Method Package Cell Culture Profiling Ver. 2

Acknowledgement

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<References>

1) Ryan J. Graham et al., "Consequences of trace metal variability and supplementation on Chinese hamster ovary (CHO) cell culture performance: A review of key mechanism and considerations," Biotechnology and Bioengineering, 2019
2) Prabhu et al., "Zinc supplementation decreases galactosylation of recombinant IgG in CHO cells," Applied Microbiology and Biotechnology, 2018
3) Application News A634 "Direct Analysis of Metallic Elements in Cell Culture Medium by Atomic Absorption Spectrophotometry (AAS)"
4) Application News A651 "Monitoring of Metal Elements in Cell Culture Supernatant using Atomic Absorption Spectrophotometer"
5) Application News 01-0372 "Analysis of Metal Elements in Culture Medium Using ICPMS -2030"

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