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Application News

Inductively Coupled Plasma Mass Spectrometer ICPMS-2030

Analysis of Metal Elements in Culture Medium Using ICPMS-2030

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User Benefits

- Simultaneous analysis is possible for metal elements in culture medium.
- Culture medium can be analyzed with only dilution using the ICPMS-2030.
- ICPMS-2030 can perform accurate analysis of metal elements even in high-matrix medium.

■ Introduction

It has been reported that metal elements in culture medium are important in cell culture because they are taken up by cells and contribute to enzyme reactions and redox reactions. On the other hand, there is a risk of contamination of metal elements from raw materials and equipment in the culture medium manufacturing process, so the concentration of metal elements may differ among lots. Therefore, it is important to know the concentration profile of metal elements in the medium to realize reproducible cell culture.[1)]

We measured multiple elements, mainly essential trace elements, using the ICPMS-2030, which can perform sensitive analysis of multiple elements simultaneously. In ICP-MS measurements, inorganic salts and organic compounds (matrices) contained in the medium may cause interference and interface clog, affecting analytical values. In this Application News, we evaluated the spike recovery rates and long-term stability in culture medium using the ICPMS-2030.

■ Measuring Elements

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

■ Samples

1. Preparation of high–matrix medium

   In order to evaluate the spike recovery rate and long-term stability of the analysis, a high-matrix medium was prepared by adding purified protein at a concentration of 10 g/L to DMEM (+4.5 g/L glucose), which has one of the highest concentrations of inorganic salts among the media. (The composition is disclosed.)

2. Other commercially available media

   We prepared a representative culture media for each cell type (CHO cells, iPS/ES cells, T cells, and mesenchymal stem cells) as an example of the measurement. (The composition is not available.)

■ Pretreatment of Samples

1. For spike recovery test

   • Unspiked samples

   Unspiked samples were prepared by diluting culture media 20 times with 1 v/v% nitric acid.

   • Spiked samples

   Spiked samples were prepared by adding single-element standard solutions and diluting culture media 20 times with 1 v/v% nitric acid.

2. For long-term stability test

   Samples for the evaluation of long-term stability were prepared by adding single-element standard solutions to a high-matrix medium to make 20 µg/L of each element and diluting them with 1 v/v% nitric acid. Both 20 times and 100 times diluted solutions were prepared.

■ Calibration Solutions

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

Table 1 Concentration of Elements in Calibration Solutions

Elements	Calibrations Solutions (µg/L)					
	STD0	STD1	STD2	STD3	STD4	STD5
Co	0	1	5	20		
Cu	0	0.5	2.5	10		
Fe	0	5	25	100	1000	2000
Mg	0	500	2500	10000		
Mn	0	1	5	20		
Mo	0	0.1	0.5	2		
Ni	0	0.25	1.25	5		
Se	0	0.25	1.25	5		
Zn	0	10	50	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

A spike recovery test of a high-matrix medium was performed to confirm accuracy with the media. The results are shown in Table 3. The spike recovery rates for all elements were within ± 10 %. Based on this result, low interferences were observed for measurements of media by the ICPMS-2030.

■ Long-Term Stability Test

A high-matrix medium was measured 50 times to evaluate long-term stability. The results are shown in Fig. 1 to 4.

In the 20 times diluted solution, the intensity of each internal standard element decreased by about 30 % (Fig. 1). The interface gradually became clogged. However, the variation of analytical values of each element by the internal standard method was within 95–107 % (Fig. 2).

In the analysis of the 100 times diluted solution, there were small variations in the intensity of each internal standard element (Fig. 3) and in the analytical values of each element (Fig. 4).

■ Measurement of Culture Media for Each Cell Type

Typical culture media (composition not available) used for the culture of each cell type (CHO cells, iPS/ES cells, T cells, mesenchymal stem cells) were measured, also spike recovery tests were performed. These results are shown in Table 4. 90 to 110 % recoveries are obtained in all culture media.

Table 3 Spike Recovery Test of High-Matrix Medium

Unit : µg/L

Elements	Co	Cu	Fe	Mg	Mn	Mo	Ni	Se	Zn
Detection Limit	**0.006**	**0.04**	**0.01**	**0.08**	**0.01**	**0.007**	**0.008**	**0.01**	**0.02**
Spike Conc.	1	0.5	5	500	1	0.1	0.25	0.25	10
Unspiked Sample	0.02	1.27	0.84	940	0.05	0.039	0.103	0.13	1.9
Spiked Sample	1.03	1.72	5.78	1430	1.03	0.148	0.359	0.39	12.0
Recovery Rate (%)	**101**	**90**	**99**	**98**	**98**	**109**	**102**	**104**	**101**

Recovery Rate (%) = (Spiked Sample – Unspiked Sample)/Spike Conc. × 100
Detection Limit = 3σ (σ: standard deviation of calibration solution's blank)

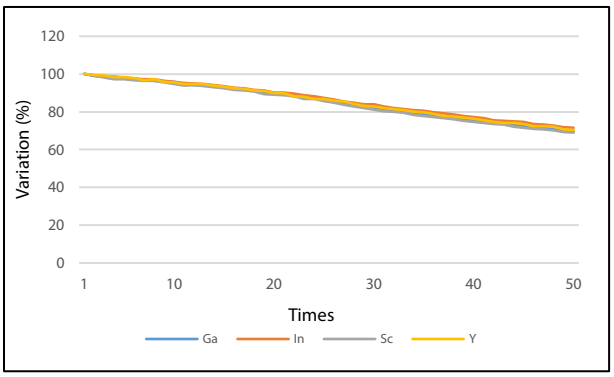

Fig. 1 Intensity Variation of Each Internal Standard Element (20 Times Dilution)

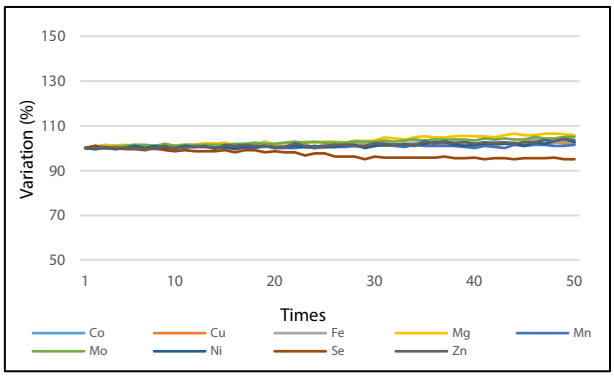

Fig. 2 Variation of Analytical Values (20 Times Dilution)

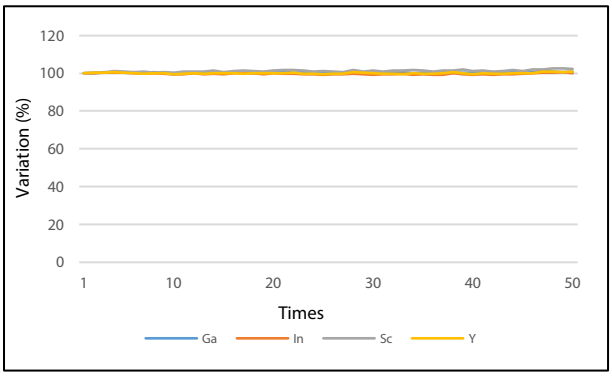

Fig. 3 Intensity Variation of Each Internal Standard Element (100 Times Dilution)

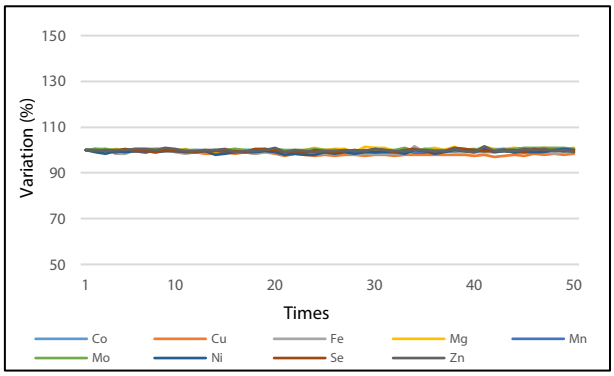

Fig. 4 Variation of Analytical Values (100 Times Dilution)

Table 4 Spike Recovery Test of Culture Media for Each Cell Type

Unit: µg/L

Culture Medium	Elements	Co	Cu	Fe	Mg	Mn	Mo	Ni	Se	Zn
	Detection Limit	0.006	0.04	0.01	0.08	0.01	0.007	0.008	0.01	0.02
CHO cells	Unspiked Sample	10.0 (200)	N.D. (N.D.)	880 (17600)	1400 (28000)	1.23 (24.6)	0.010 (0.20)	0.038 (0.76)	0.12 (2.4)	26.8 (536)
	Spiked Sample	14.7	0.52	1880	1900	2.20	0.107	0.296	0.39	36.1
	Spike Conc.	5	0.5	1000	500	1	0.1	0.25	0.25	10
	Recovery Rate (%)	**94**	**104**	**100**	**100**	**97**	**97**	**103**	**108**	**93**
iPS/ES cells	Unspiked Sample	1.20 (24.0)	0.08 (1.6)	3.99 (79.8)	1020 (20400)	0.01 (0.2)	0.015 (0.30)	0.029 (0.58)	0.24 (4.8)	7.7 (154)
	Spiked Sample	2.21	0.57	8.86	1540	0.96	0.113	0.278	0.51	17.6
	Spike Conc.	1	0.5	5	500	1	0.1	0.25	0.25	10
	Recovery Rate (%)	**101**	**98**	**97**	**104**	**95**	**98**	**100**	**108**	**99**
T cells	Unspiked Sample	0.03 (0.6)	0.21 (4.2)	2.26 (45.2)	910 (18200)	0.05 (1.0)	0.025 (0.50)	0.076 (1.52)	0.24 (4.8)	4.5 (90)
	Spiked Sample	1.02	0.69	7.18	1400	1.03	0.127	0.301	0.50	14.6
	Spike Conc.	1	0.5	5	500	1	0.1	0.25	0.25	10
	Recovery Rate (%)	**99**	**96**	**98**	**98**	**98**	**102**	**90**	**104**	**101**
Mesenchymal stem cells	Unspiked Sample	2.00 (40.0)	0.10 (2.0)	0.14 (2.8)	880 (17600)	0.01 (0.2)	0.034 (0.68)	0.054 (1.08)	0.13 (2.6)	0.03 (0.6)
	Spiked Sample	3.01	0.57	5.08	1390	0.97	0.127	0.305	0.38	9.88
	Spike Conc.	1	0.5	5	500	1	0.1	0.25	0.25	10
	Recovery Rate (%)	**101**	**94**	**99**	**102**	**96**	**93**	**100**	**100**	**99**

Recovery Rate (%) = (Spiked Sample – Unspiked Sample)/Spike Conc. × 100
Detection Limit = 3σ (σ: standard deviation of calibration solution's blank)
N.D.: below the detection limit
(): the data is converted to the concentration in the culture medium

■ Conclusion

In this report, the ICPMS-2030 was used for simultaneous measurements of multiple metal elements in culture media with only dilution. Good results were obtained in spike recovery tests of high-matrix medium. Therefore, it was found that accurate analysis was possible even in high-matrix medium. In long-term stability tests of 20 times diluted solution, the intensity of the internal standard elements decreased; however, the value measured by the internal standard method did not fluctuate greatly. When a high-matrix medium is analyzed continuously for a long period of time, it is considered that the analysis values are more stable and the maintenance frequency, such as cleaning of the interface, can be reduced by analyzing at a higher dilution ratio, like the 100 times dilution analysis in this Application News.

The spike recovery test of culture media for each cell type also showed good results. It was found that culture media of various cell types could be measured accurately.

Inductively Coupled Plasma Mass Spectrometer ICPMS-2030

■ 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 supernatant. 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

<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

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