Application News

Inductively Coupled Plasma Mass Spectrometer ICPMS-2050

Analysis of Metal Elements in Culture Medium Using ICPMS-2050

Kosuke Naka[1], Yujing Jiang[1], Hirotaka Kuroda[1, 2, 3]
1 Shimadzu Corporation, 2 Shimadzu Omics Innovation Research Laboratories,
3 Osaka University Graduate School of Engineering Department of Biotechnology

User Benefits

- Analysis of both trace and high-concentration elements is possible in culture media.
- Culture media can be analyzed with simple dilution-only pretreatment using the ICPMS-2050.
- The elements in the culture medium can be analyzed reliably and accurately over a long period.

■ Introduction

A culture medium usually contains elements spanning a wide range of concentrations, from trace (Co, Cu, Se, etc.) to several thousand ppm (Na). Elements in the medium are important in cell cultures because they are taken up by the cells and contribute to enzyme activity and redox reactions. On the other hand, it has been pointed out that unexpected contamination from raw materials and equipment in the culture media manufacturing process may cause differences in element concentrations among batches. Therefore, it is important to know the element concentration profiles in the culture medium to achieve a homogeneous cell culture.

ICP-MS is suitable for this purpose because it can analyze multiple elements with high sensitivity and has a wide dynamic range. In this Application News, an ICPMS-2050 was used to analyze both trace and high-concentration elements in a culture medium by dilution-only pretreatment. Furthermore, the spike recovery and long-term stability in the culture medium were evaluated using the ICPMS-2050 to confirm that there was no effect on the analytical value due to dilution-only pretreatment.

■ Analysis Elements

Trace elements: Ag, Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ge, Li, Mn, Mo, Ni, Se, Sn, Ti, V, Zn

High-concentration elements: Ca, K, Mg, Na, P

■ Samples and Pretreatment

- Analytical Samples

DMEM (high glucose) was used as an analytical sample. In addition, to evaluate the influence of proteins produced during the cultivation process on the analysis, a high-matrix medium was prepared by adding purified protein at a concentration of 10 g/L to DMEM (Hereafter referred to as high matrix medium).

- Pretreatment

Unspiked samples: Unspiked samples were prepared by diluting culture media 20 times with mixed acid (1v/v% nitric acid and 0.5v/v% hydrochloric acid).

Spiked samples: Spiked samples were prepared by adding single-element standard solutions and diluting the culture media 20 times with mixed acid (1v/v% nitric acid and 0.5v/v% hydrochloric acid).

■ Standard Samples

- Calibration Standards

Calibration curve standards were prepared by mixing commercially available single-element standard solutions and diluting them with mixed acid (1v/v% nitric acid and 0.5v/v% hydrochloric acid). The concentrations in each calibration curve standard are shown in Table 1.

- Internal Standard Solution

The internal standard solution was prepared by mixing commercially available single-element standard solutions (Be, Ga, Sc, Rh, Tl) and diluted with mixed acid (1v/v% nitric acid and 0.5v/v% hydrochloric acid). The concentrations of Be, Ga, and Sc in the internal standard solution were 1 mg/L, and the concentrations of Rh and Tl were 0.2 mg/L.

- Continuing Calibration Verification (CCV) Sample

CCV was prepared at the same concentration as STD3.

Table 1 Calibration Curve Standards

Elements	Unit	Concentration					
		STD0	STD1	STD2	STD3	STD4	STD5
Ag, Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ge, Li, Mn, Mo, Ni, Se, Sn, V, Zn	μg/L	0	0.5	1	5	10	
Ti	μg/L	0	5	10	50	100	
Ca, Mg, P	mg/L	0		1	5	10	
K	mg/L	0		2.5	12.5	25	
Na	mg/L	0			30	60	300

■ Equipment Configuration and Analytical Conditions

The configuration of the ICP-MS system is shown in Table 2. To reduce running costs, the analysis was performed using a mini-torch, which consumes less argon gas than a normal plasma torch. To save labor in sample preparation, the internal standards were added online using an online internal standard kit.

Table 3 shows the analytical conditions.

Table 2 ICP-MS System Configuration

System:	ICPMS-2050
Nebulizer:	Nebulizer DC04
Chamber:	Cyclone Chamber
Torch:	Mini-Torch
Skimmer Cone:	Nickel
Sampling Cone:	Nickel
Autosampler:	AS-20
Internal Standard Elements:	Online Internal Standard Kit (sample: internal standard = about 9: 1)

Table 3 Analytical Conditions

RF Power:	1.20 kW
Sampling Depth:	7.0 mm
Plasma Gas Flowrate:	9.0 L/min
Auxiliary Gas Flowrate:	1.10 L/min
Carrier Gas Flowrate:	0.60 L/min
Dilution Gas Flowrate:	0.25 L/min
Pump Speed:	15 r.p.m.
Collision / Reaction Gas:	He / H_2

■ Sample Analysis and Spike Recovery

Analytical samples were quantitatively analyzed using the calibration curves shown in Table 1.

Table 4 shows the quantification results for the solution and the culture medium. The results for the culture medium were calculated by dilution factor. It was found that the simultaneous analysis of multiple elements in the culture medium that are present in a wide range of concentrations, from sub-ppb to several thousand ppm, is possible.

Furthermore, DMEM and high matrix media spike recovery tests were conducted to demonstrate that dilution-only pretreatment had no effect on the analytical values. The spike recoveries were calculated, and the results are shown in Table 5. Spike recoveries of 94 to 107% were obtained for all the measured elements, showing that the culture medium can be accurately analyzed with dilution-only pretreatment using the ICPMS-2050.

Table 4 Quantification Results in the Solution and in the Culture Medium

Elements		Collision / Reaction Gas	Internal Standard Element	Instrument Detection Limit (IDL)*	DL in Sample**	DMEM		High Matrix Medium	
						Mean Measured Value in Solution	Mean Measured Value in Sample	Mean Measured Value in Solution	Mean Measured Value in Sample
Trace Elements (μg/L)	^{107}Ag	He	^{103}Rh	0.005	0.1	N.D.***	N.D.	N.D.	N.D.
	^{27}Al	No Gas	^{45}Sc	0.05	1	0.06	1	4.57	91.4
	^{138}Ba	He	^{103}Rh	0.005	0.1	0.016	0.32	0.517	10.3
	^{209}Bi	He	^{205}Tl	0.002	0.04	N.D.	N.D.	0.003	0.06
	^{111}Cd	He	^{103}Rh	0.01	0.2	N.D.	N.D.	N.D.	N.D.
	^{59}Co	He	^{71}Ga	0.01	0.2	N.D.	N.D.	0.06	1
	^{52}Cr	He	^{71}Ga	0.02	0.4	0.02	0.4	0.17	3.4
	^{63}Cu	He	^{71}Ga	0.006	0.1	0.007	0.1	0.599	12.0
	^{56}Fe	H_2	^{71}Ga	0.01	0.2	0.73	15	3.87	77.4
	^{72}Ge	He	^{71}Ga	0.03	0.6	N.D.	N.D.	N.D.	N.D.
	^{7}Li	No Gas	^{9}Be	0.003	0.06	0.007	0.1	0.020	0.40
	^{55}Mn	He	^{71}Ga	0.02	0.4	N.D.	N.D.	0.05	1.0
	^{98}Mo	He	^{103}Rh	0.007	0.1	N.D.	N.D.	0.022	0.44
	^{60}Ni	He	^{71}Ga	0.02	0.4	0.02	0.4	0.40	8.0
	^{78}Se	H_2	^{71}Ga	0.01	0.2	N.D.	N.D.	0.07	1
	^{118}Sn	He	^{103}Rh	0.02	0.4	N.D.	N.D.	N.D.	N.D.
	^{47}Ti	He	^{45}Sc	0.5	10	N.D.	N.D.	N.D.	N.D.
	^{51}V	He	^{45}Sc	0.03	0.6	N.D.	N.D.	N.D.	N.D.
	^{66}Zn	He	^{71}Ga	0.02	0.4	N.D.	N.D.	0.11	2.2
High-Concentration Elements (mg/L)	^{44}Ca	He	^{45}Sc	0.05	1	3.52	70.4	3.48	69.6
	^{39}K	He	^{45}Sc	0.008	0.2	10.7	214	10.6	212
	^{24}Mg	He	^{45}Sc	0.0008	0.02	0.971	19.4	0.965	19.3
	^{23}Na	He	^{45}Sc	0.04	0.8	178	3560	177	3540
	^{31}P	He	^{45}Sc	0.02	0.4	1.44	28.8	1.40	28.0

*IDL: 3 × σ (standard deviation of calibration solution's blank) × slope of the calibration curve
**DL in Sample: IDL × dilution times (20 times)
***N.D.: below the detection limit

Table 5 Result of Spike Recovery

Elements		Instrument Detection Limit (IDL)*	Spike Concentration	DMEM	Spiked DMEM		High Matrix Medium	Spiked High Matrix Medium	
				Mean Measured Value in Solution	Mean Measured Value in Solution	Recovery (%)	Mean Measured Value in Solution	Mean Measured Value in Solution	Recovery (%)
Trace Elements (μg/L)	^{107}Ag	0.005	5	N.D.**	4.93	99	N.D.	4.94	99
	^{27}Al	0.05	5	0.06	5.06	100	4.57	9.93	107
	^{138}Ba	0.005	5	0.016	5.10	102	0.517	5.72	104
	^{209}Bi	0.002	5	N.D.	4.90	98	0.003	4.91	98
	^{111}Cd	0.01	5	N.D.	4.91	98	N.D.	4.92	98
	^{59}Co	0.01	5	N.D.	4.96	99	0.06	4.98	98
	^{52}Cr	0.02	5	0.02	5.09	101	0.17	5.33	103
	^{63}Cu	0.006	5	0.007	4.85	97	0.599	5.39	96
	^{56}Fe	0.01	5	0.73	5.65	98	3.87	8.98	102
	^{72}Ge	0.03	5	N.D.	4.98	100	N.D.	4.94	99
	^{7}Li	0.003	5	0.007	4.98	98	0.020	4.74	94
	^{55}Mn	0.02	5	N.D.	5.02	100	0.05	5.16	102
	^{98}Mo	0.007	5	N.D.	5.12	102	0.022	5.11	102
	^{60}Ni	0.02	5	0.02	4.92	98	0.40	5.28	98
	^{78}Se	0.01	5	N.D.	4.95	99	0.07	5.36	106
	^{118}Sn	0.02	5	N.D.	5.03	101	N.D.	5.07	101
	^{47}Ti	0.5	50	N.D.	50.8	101	N.D.	50.6	101
	^{51}V	0.03	5	N.D.	4.98	100	N.D.	4.90	98
	^{66}Zn	0.02	5	N.D.	4.83	97	0.11	4.99	98
High-Concentration Elements (mg/L)	^{44}Ca	0.05	5	3.52	8.51	100	3.48	8.52	101
	^{39}K	0.008	12.5	10.7	23.0	98	10.6	23.4	102
	^{24}Mg	0.0008	5	0.971	5.84	97	0.965	5.91	99
	^{23}Na	0.04	100	178	277	99	177	283	106
	^{31}P	0.02	5	1.44	6.56	102	1.40	6.67	105

*IDL: 3 × σ (standard deviation of calibration solution's blank) × slope of the calibration curve
**N.D.: below the detection limit

■ Long-Term Stability

Both DMEM and high matrix media were analyzed for approximately 8 hours to evaluate the long-term stability of the ICPMS-2050. CCV was measured every 10 samples (100 samples in total) to confirm the validity of the calibration curves. Recoveries of CCV during the analysis are shown in Fig. 1.

The CCV recoveries of all the measured elements during the analysis were within 90 to 110 % (red dotted line), which confirmed the validity of the calibration curve during the long-term analysis.

In addition, the internal standard recoveries during analysis are shown in Fig. 2. The intensity of each internal standard element in STD0 was defined as 100 %. All measured internal standard recoveries were within 70 to 130 % (red dotted line). The CCV and internal standard recoveries showed good long-term stability of ICPMS-2050 analysis.

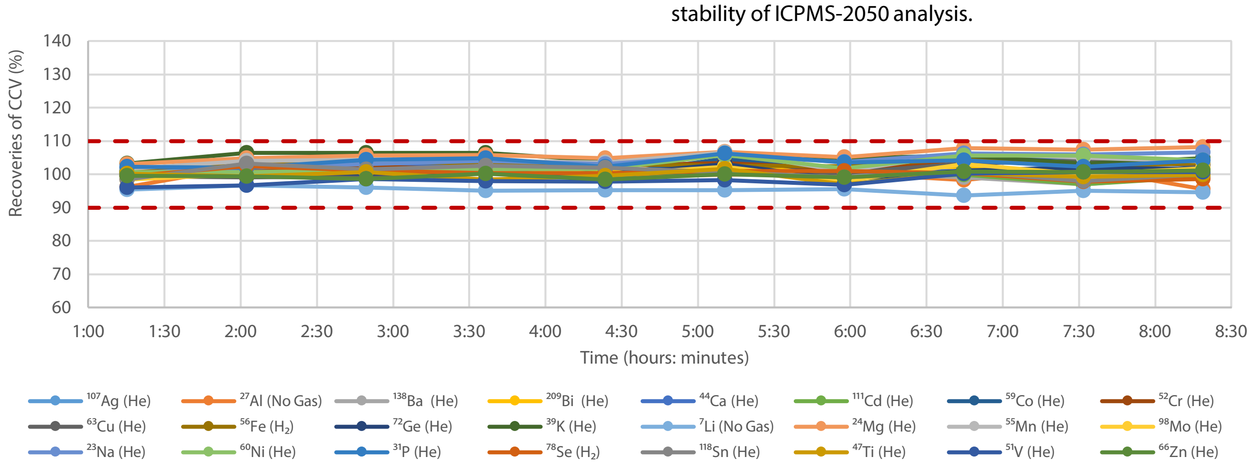


Fig. 1 CCV Recoveries over 8 Hours Analysis

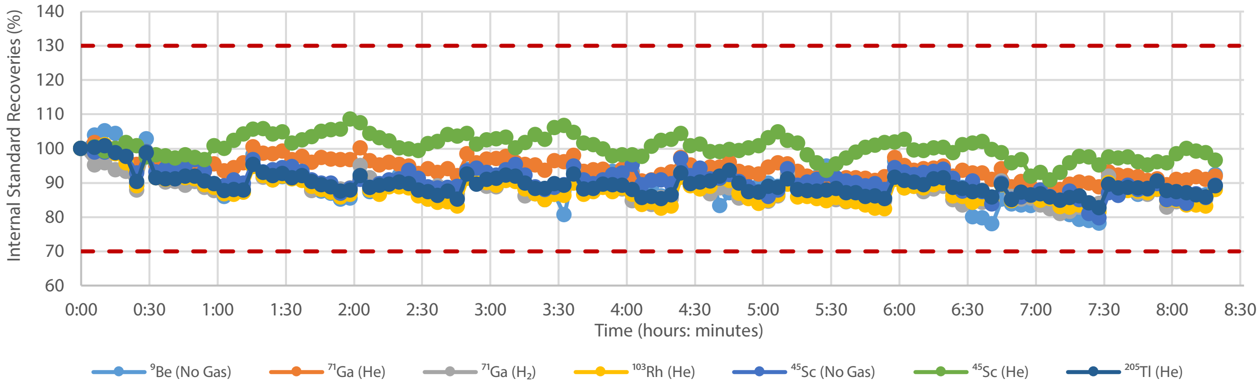
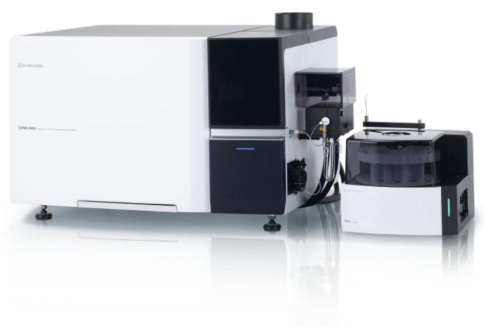


Fig. 2 Internal Standard Recoveries over 8 Hours Analysis

■ Summary

In this Application News, the ICPMS-2050 was used to analyze trace and high-concentration elements in a culture medium using dilution-only pretreatment. Good spike recoveries were obtained for all the measured elements, verifying that the culture medium can be accurately analyzed with dilution-only pretreatment. In addition, the results of CCV and internal standard recoveries showed good long-term stability.



<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

<Related Applications>

Culture Medium Analysis for a Metabolic Analysis of Antibody-Producing Cells Using LC-MS/MS and ICP-MS
Application News 01-00498

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

01-00712-EN First Edition: Mar. 2024