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Application News

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Atomic Absorption Spectrophotometer AA-7000F / AAC / GFA-7000A / ASC-7000

Monitoring of Metal Elements in Cell Culture Supernatant using Atomic Absorption Spectrophotometer

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User Benefits

- The cell culture supernatant can be analyzed with only dilution using the atomic absorption spectrophotometer.
- Using the auto atomizer changer (AAC), which can easily switch between the flame method and electric thermal method, it is possible to easily analyze a wide range of metal elements from trace amounts (ppb) to high concentrations (ppm).

■ 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 cellular metabolism and primary structure of the antibodies produced in a culture medium are influenced by the metallic element concentration of the medium. For example, where metallic elements in the cell culture supernatant are concerned, it has been noted that the pathway of lactic acid metabolism in CHO cells changes depending on the copper (Cu) concentration in the culture medium[1], and the constituent sugars of glycans attached to IgG antibodies change depending on the Mn/Zn ratio in the culture medium[2]. Therefore, monitoring the concentrations of metallic elements in the cell culture supernatant is considered critical for maintaining uniform quality in antibody drugs.

In previous studies, we have presented the case of analyzing metal elements in a cell-free pre-culture medium using atomic absorption spectroscopy with only dilution pretreatment[3]. In this study, we verify that this method can be applied to the analysis of metal elements in the medium (culture supernatant) collected over time during cell culture.

■ Cell Culture and Preparation of Samples

CHO cells were shake-cultured for 4 days in a 125 mL culture flask (120 rpm). After collecting 1 mL of the culture solution immediately after the start of culture, the same amount was collected every 24 h. After centrifuging each collected culture solution (5 min, 4℃), the culture supernatant sample was prepared by dispensing the culture supernatant into a separate tube.

Figure 1 shows the procedure for cell culture and sample preparation.

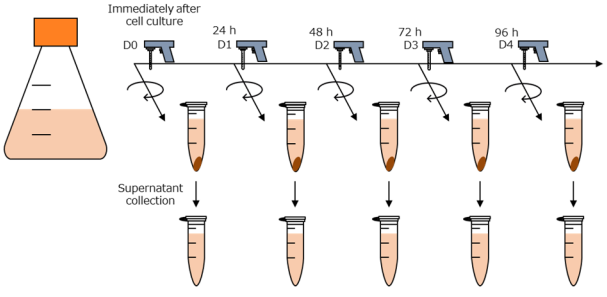

Fig. 1 Cell culture and preparation of samples

■ Instrument and Analysis Conditions

The instrument used in the analysis was a Shimadzu AA-7000 atomic absorption spectrophotometer with a graphite furnace atomizer (automatic switching between the flame method and electric thermal method is possible) and an autosampler.

Regarding the atomization method, the flame method was used for the elements (Mg and Zn) known to be contained in the culture solution at high concentration, and the electric thermal method was used for the other trace elements (Cu, Mn, Co, and Fe).

Table 1 and 2 list the main spectrophotometry and atomization conditions in measurements performed by the flame method and electric thermal method, respectively.

Table 1 Analysis conditions of the flame method

Element	Zn	Mg
Analysis Wavelength	213.9 nm	285.2 nm
Slit Width	0.7 nm	
Lighting Mode	BGC-D2	
Lamp Current	8 mA	
Flame Type	Air−Acetylene	
C_2H_2 Flow Rate	2.0 L/min	1.8 L/min
Integration Time	3 s	
Repetition	3 times	

Table 2 Analysis conditions of the electric thermal method

Element	Cu	Mn	Co	Fe
Analysis Wavelength	324.8 nm	279.5 nm	240.7 nm	248.3 nm
Slit Width	0.7 nm	0.2 nm	0.2 nm	0.7 nm
Lighting Mode	BGC-D2			
Lamp Current	8 mA	10 mA	12 mA	12 mA
Ashing Temp.	800 ℃			
Atomization Temp.	2500 ℃	2200 ℃	2300 ℃	2300 ℃
Tube Type	Platform Tube			
Injection Volume	5 µL	10 µL		
Signal Processing	Height	Area	Height	
Repetition	2 times (max 3 times)			

■ Analysis Method

・Each Sample:

Diluted for each measurement element.

(The nitric acid concentration is 0.07 mol/L (0.5 v/v%))

・Calibration curve standard solution:

Prepared by diluting 1000 mg/L standard solution.

(The nitric acid concentration is 0.07 mol / L (0.5 v / v%))

■ Measurement Results

1) Flame method

Table 3 lists the measurement results of each sample. Only the medium is designated as D0, and the supernatants from the first day to the fourth day of culture are denoted as D1 to D4.

Figure 2 and 3 show the calibration curve of Zn and Mg and the change over time in concentration in the culture supernatant.

Table 3 Measurement result of the flame method

		Zn	Mg
Dilution		20 times	500 times
D0	Actual Conc.(mg/L)*1	5.2	53
	SD*2	0.039	0.29
	%RSD*3	0.74	0.54
D1	Actual Conc.(mg/L)	5.2	50
	SD	0.065	0.08
	%RSD	1.3	0.16
D2	Actual Conc.(mg/L)	4.5	48
	SD	0.072	0.43
	%RSD	1.6	0.90
D3	Actual Conc.(mg/L)	4.3	47
	SD	0.060	0.07
	%RSD	1.4	0.14
D4	Actual Conc.(mg/L)	4.1	53
	SD	0.042	0.21
	%RSD	1.0	0.45

*1 Value obtained by converting the measurement value to one corresponding to stock solution of cell culture supernatant.
*2 Standard deviation of actual conc.(mg/L) for repeated measurements.
*3 Relative standard deviation of the actual conc.(mg/L) of repeated measurements. (%RSD = SD ÷ actual conc. x 100)

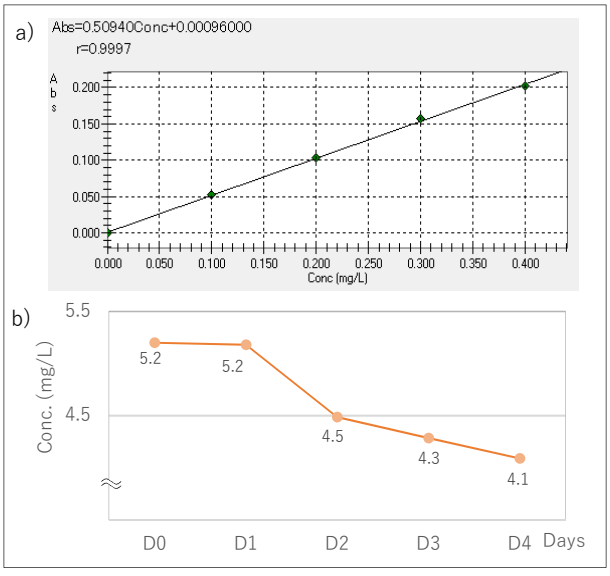

Fig. 2 Calibration curve of Zn (a) and time course of culture supernatant metal concentrations (b)

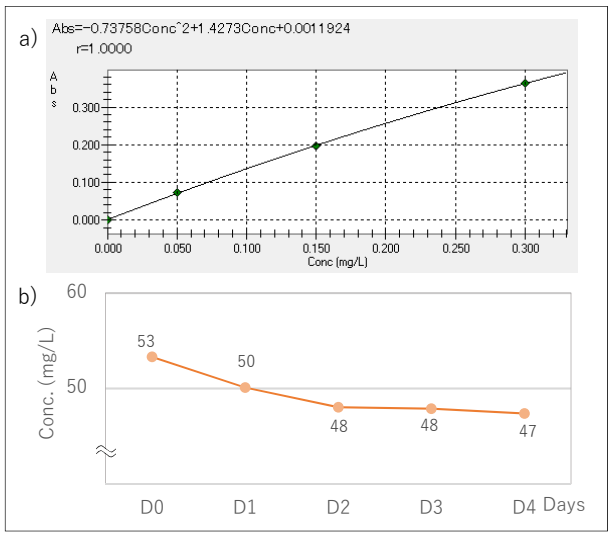

Fig. 3 Calibration curve of Mg (a) and time course of culture supernatant metal concentrations (b)

2) Electric thermal method

Table 4 summarizes the measurement results and recovery rate of each sample. Figures 4 to 7 show the calibration curve of each element and the change over time in concentration in the culture supernatant. The recovery test was carried out by adding a standard solution with a constant concentration for each element. As a result, most of them were within 100±10%, which indicated a good recovery rate.

For reference, Fig. 8 shows an excerpt of the peak profile corresponding to measurements performed by the electric thermal method. Since the peak profile of Mn differs between the standard solution and sample solution, the influence of the sample components can be inferred. Because the peak shape differs between the standard solution and the sample solution, signal processing could be used for determining the peak area only for Mn.

Table 4 Measurement results of the electric thermal method

		Cu	Mn	Co	Fe
Dilution		20 times	20 times	40 times	40 times
D0	Actual Conc. (μg/L)	16	11	250	409
	SD	0.44	0.00	6.4	2.0
	%RSD	2.8	0.00	2.6	0.49
	Recovery Rate*1	120%	101%	97%	102%
D1	Actual Conc. (μg/L)	18	12	256	420
	SD	0.15	0.82	11	1.6
	%RSD	0.83	5.2	4.3	0.38
	Recovery Rate	98%	86%	93%	102%
D2	Actual Conc. (μg/L)	14	9	259	387
	SD	0.59	0.63	1.5	2.5
	%RSD	4.3	7.1	0.58	0.65
	Recovery Rate	101%	101%	99%	100%
D3	Actual Conc. (μg/L)	24	11	258	373
	SD	0.00	0.12	12	5.7
	%RSD	0.0	1.1	4.5	1.5
	Recovery Rate	106%	93%	96%	115%
D4	Actual Conc. (μg/L)	20	17	268	382
	SD	1.5	0.38	11	9.2
	%RSD	7.3	2.2	4.0	2.4
	Recovery Rate	94%	109%	95%	99%

*1 Recovery Rate=Spiked concentration divided by the concentration difference between spiked sample and non-spiked sample

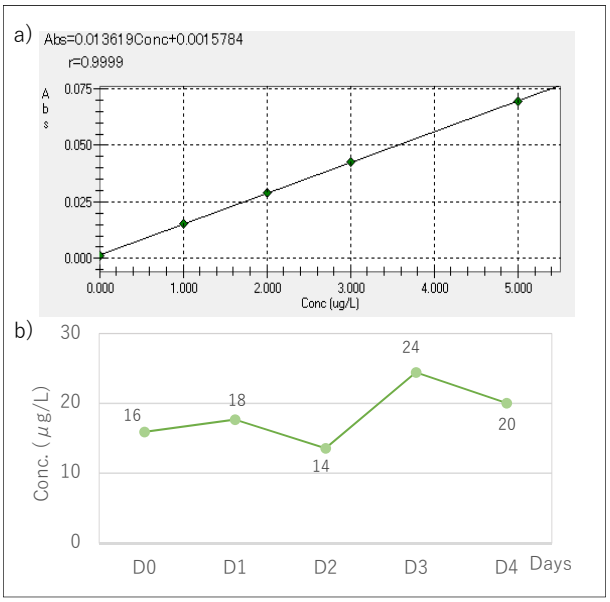

Fig. 4 Calibration curve of Cu (a) and time course of culture supernatant metal concentrations (b)

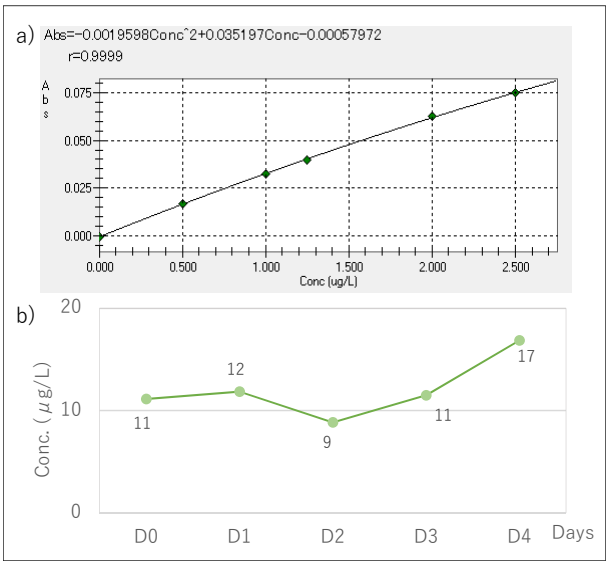

Fig. 5 Calibration curve of Mn (a) and time course of culture supernatant metal concentrations (b)

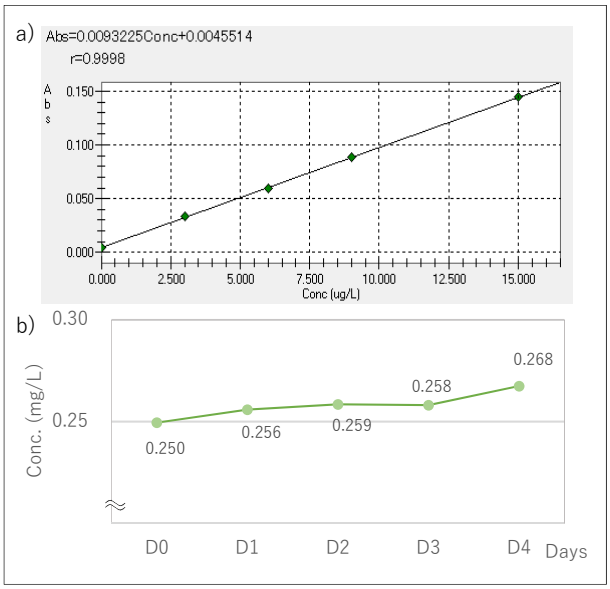

Fig. 6 Calibration curve of Co (a) and time course of culture supernatant metal concentrations (b)

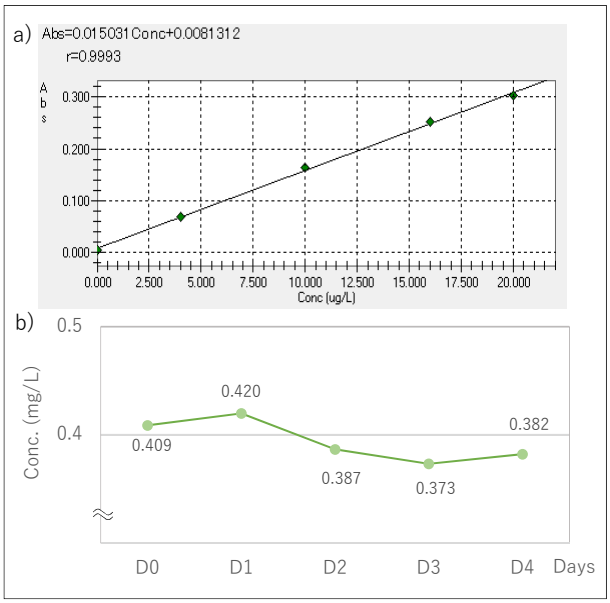

Fig. 7 Calibration curve of Fe (a) and time course of culture supernatant metal concentrations (b)

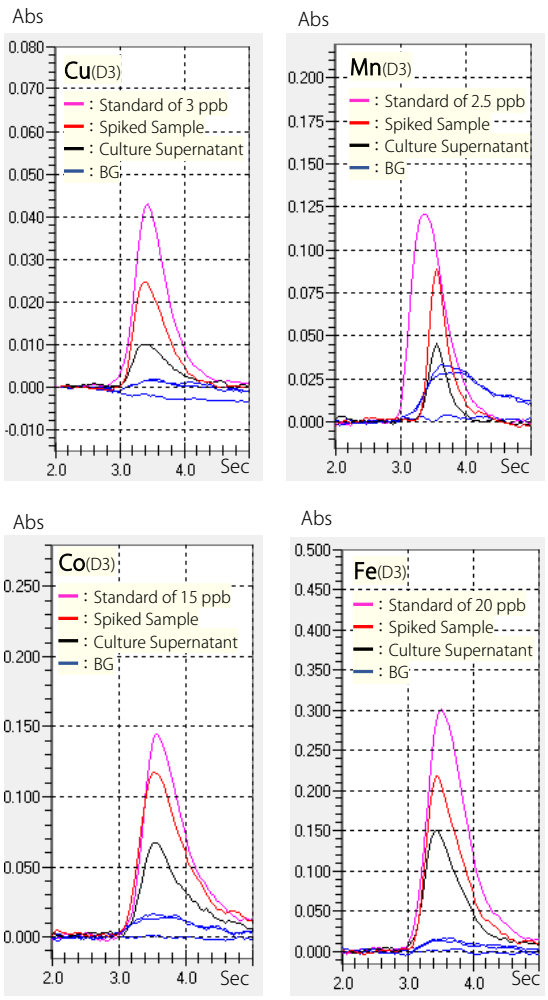

Fig. 8 Peak profile of sample

■ Conclusion

The metal element concentration in the culture supernatant was measured using the atomic absorption method (flame method/electric thermal method). As a result, it was found that analysis in a wide concentration range of several ppb to several tens of ppm is possible with a simple pretreatment that only dilutes the culture supernatant.

It was shown that the atomic absorption method can be applied to analyze not only the medium but also the culture supernatant. It can be used for monitoring the concentration of metal elements in a wide concentration range from trace amounts to high concentrations.

■ Reference

1) Inn H Yuk et al., "Effects of copper on CHO cells: Insights from gene expression analyses," Biotechnology Progress, 2014

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)

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