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Application News

No. A634

Spectrophotometric Analysis

Direct Analysis of Metallic Elements in Cell Culture Medium by Atomic Absorption Spectrophotometry (AAS)

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 the cellular metabolism and the primary structure of the antibodies produced in a culture medium are influenced by the nutrient composition (sugars, amino acids, etc.) and the metallic element concentration of the medium. For example, where metallic elements in the culture medium are concerned, it has been noted that zinc (Zn) acts a cofactor for at least 300 types of enzymes and transcription factors when absorbed into cells [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 culture medium is considered critical for maintaining uniform quality in antibody drugs.

Until now, the ICP-MS and chelate determination techniques had been used in measurements of the metallic elements in the culture media. However, ICP-MS has high initial/running costs, and the complexity of the chelate determination method is a problem, as chelate determination requires sample pretreatment for each element in order to coordinate chelating agents (chelators) with high specificity for the target elements.

Therefore, we measured the metallic elements in the culture media by atomic absorption spectrophotometry (AAS), which enables inexpensive and simple analysis of metallic elements. The content of this experiment is introduced in the following.

Y. Jiang, H. Kuroda

Principle and Features of Instrument

Atomic absorption spectrophotometry is a technique in which elements are atomized at high temperature, and the concentrations of the elements are quantified based on the fact that light of specified wavelengths is absorbed during atomization.

Atomization methods can be divided into two main types, i.e., electric heating, in which the sample is heated by passing an electric current, and flame heating by the flame of a combustible gas. Table 1 shows the extracted feature of the electric thermal method and the flame method.

In the measurements described in this article, first, an analysis was carried out by the electric thermal method, which enables high sensitivity measurement, after which elements with high concentrations in the culture medium were also evaluated by the flame method.

Table 1 Comparison of Atomization Methods

	Flame method	Electric thermal method
Sensitivity	ppb - ppm	ppt - ppb
Atomization efficiency	10 % (approx.)	90 % or more
Required sample/analysis	1 - 2 mL	5 - 50 µL
Analysis time/analysis	5 - 10 s	2 - 5 min
Repeatability	RSD 1 % (approx.)	RSD 3 % (approx.)

Samples

CHO cell culture medium (3 types*, A, B, C)

* Different vendors

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.

Table 2 and Table 3 show the main spectrophotometry and atomization conditions in measurements by the electric thermal method and flame method, respectively.

Table 2 Spectrophotometry and Atomization Conditions of Electric Thermal Method

	Analytical wavelength	Slit width	Ashing temp.	Atomization temp.	Lamp mode	Tube type
Cu	324.8 nm	0.7 nm	800 °C	2500 °C	BGC-D2	Pyro-coated tube
Mn	279.5 nm	0.2 nm		2200 °C		
Co	240.7 nm			2300 °C		
Fe	248.3 nm	0.2 nm		2300 °C		
Zn	213.9 nm	0.7 nm	400 °C	2200 °C		

Table 3 Spectrophotometry and Atomization Conditions of Flame Method

	Analytical wavelength	Slit width	Lamp mode	Flame type	Acetylene flow rate
Fe	248.3 nm	0.2 nm	BGC-D2	Air-acetylene	2.2 L/min
Zn	213.9 nm	0.7 nm			2.0 L/min

Measurement Method

Each cell culture medium was diluted as shown in Table 4 for the respective element to be measured, and the nitric acid concentration was adjusted to 0.5 w/v%.

The standard solutions for each element were prepared by diluting the 1,000 mg/L standard solution for AAS, respectively, and adjusting the nitric acid concentration to 0.5 w/v%.

All analyses were carried out by the calibration curve method.

Measurement Results

Table 5 shows the measurement results and the recovery rate for each cell culture medium. In the spike recovery test, a standard solution of a certain concentration was added to each element and concentration measurements were conducted. The recovery rate was obtained by dividing the difference between the concentrations of the spiked and unspiked samples by the spike concentration.

Because similar results were obtained for the Fe and Zn concentrations in the cell culture medium by the electric thermal method and the flame method, the measurement results of the flame method will be presented here as an example.

The value of $10\,\sigma_{bL}$ is shown as a guideline for the limit of quantitation (LOQ). This value was calculated from the standard deviation (SD) obtained by 10 repeated measurement of the culture medium, which contains substantially no metallic elements.

Fig. 1 and Fig. 2 show the calibration curves obtained by the electric thermal method and the flame method, respectively. Satisfactory correlation coefficients of r = 0.999 or higher were obtained for all calibration curves.

Fig. 3 shows the extracted peak profiles for measurement of culture medium B by the electric thermal method.

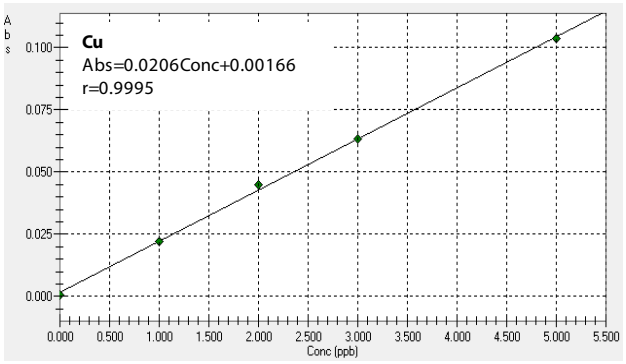
Table 4 Dilution Factors for Cell Culture Medium

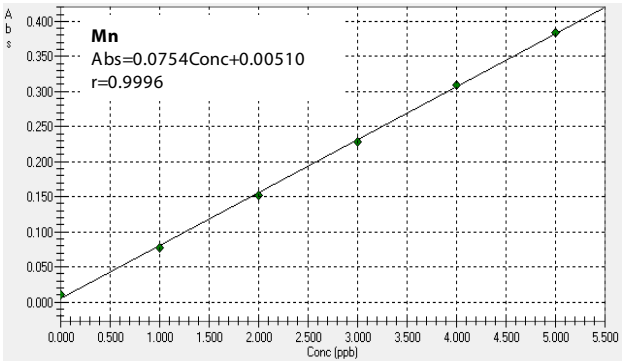
	Electric thermal method					Flame method	
	Cu	Mn	Co	Fe	Zn	Fe	Zn
A	10×	20×	10×	2000×	200×	10×	2×
B		10×	20×	500×	500×	2×	
C			10×		1000×		

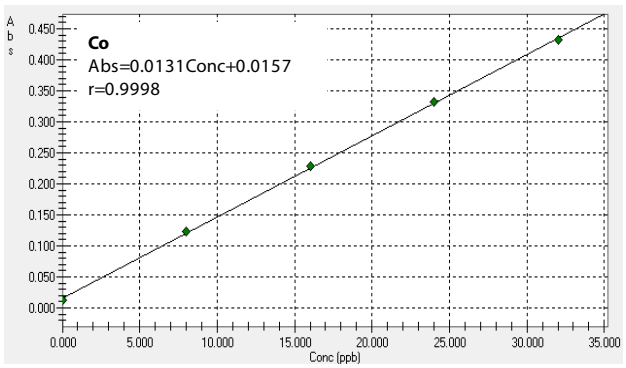
Table 5 Measurement Results of Cell Culture Media

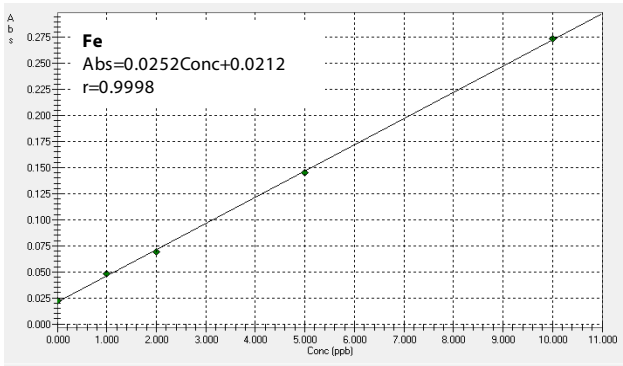
		Electric thermal method (µg/L)			Flame method (mg/L)	
		Cu	Mn	Co	Fe	Zn
A	Actual concentration *1	<5 *2	28	218	19	0.47
	Recovery rate	128 %	113 %	93 %	100 %	95 %
B	Actual concentration *1	<5 *2	<4 *2	467	3.4	0.73
	Recovery rate	93 %	110 %	106 %	107 %	93 %
C	Actual concentration *1	<5 *2	<4 *2	37.8	1.5	0.34
	Recovery rate	93 %	118 %	91 %	112 %	101 %

*1 Value obtained by converting the measurement value to the stock solution of cell culture medium.
*2 Indicates limit of quantitation (LOQ) or lower.









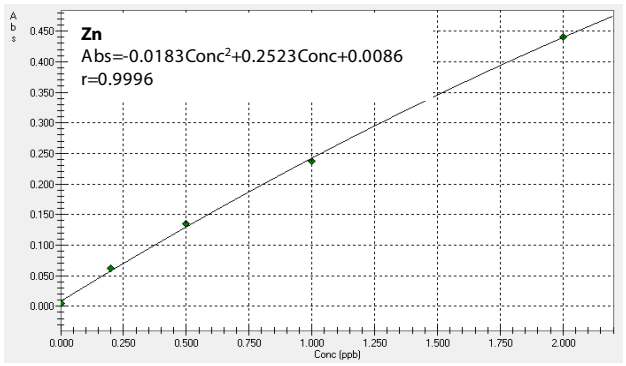
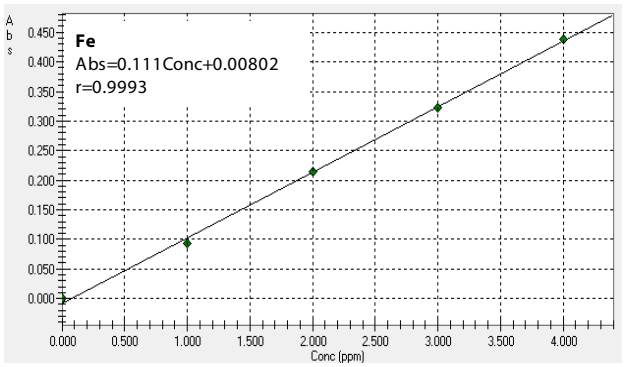


Fig. 1 Calibration Curves of Electric Thermal Method



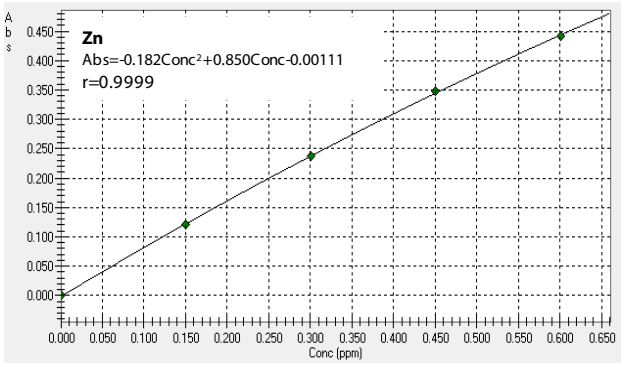


Fig. 2 Calibration Curves of Flame Method

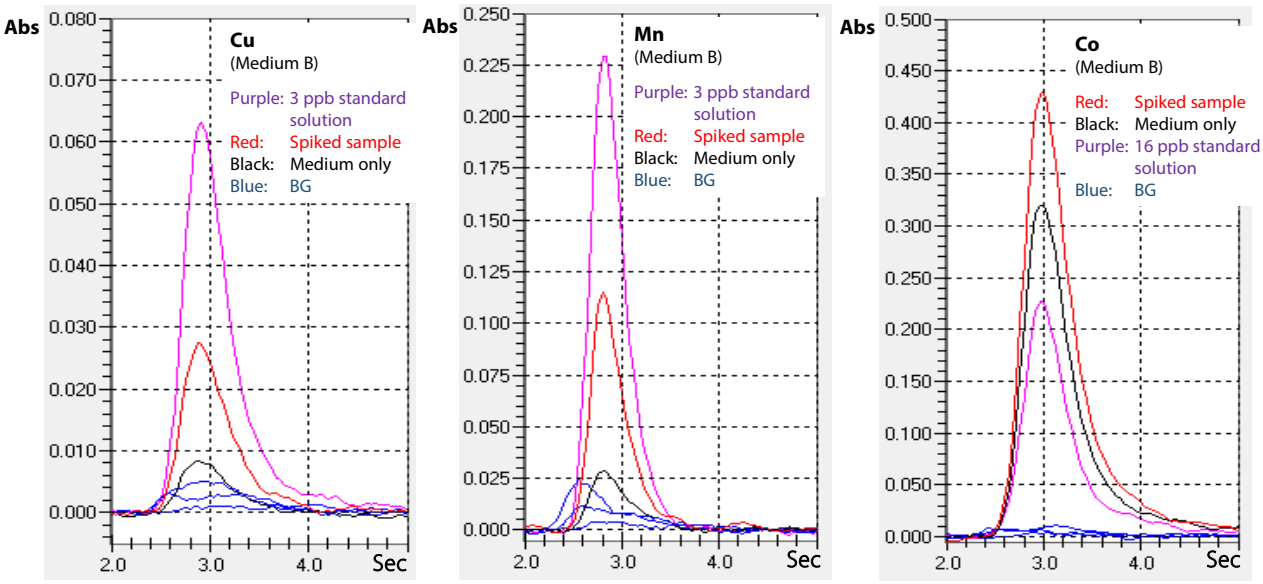


Fig. 3 Extracted Peak Profiles of Sample Measurement by Electric Thermal Method

Conclusion

The concentrations of metallic elements in three types of CHO cell culture media were measured by atomic absorption spectrophotometry (AAS; electric thermal method, flame method). This experiment demonstrated the possibility of measuring metallic elements by AAS with simple sample preparation consisting of only dilution of the culture medium. Differences were detected in the concentrations of designated metallic elements in the CHO cell culture media obtained from each vender.

Based on these results, it is clear that AAS is a simple technique for measurement of metallic elements in the culture media.

Although the recovery rates in the spike recovery tests in this experiment were generally satisfactory, there is a possibility that the mineral composition or presence of organic matter may have affected the recovery rate, depending on the medium. In that case, improvement may be possible by changing the dilution factor or changing the quantitative method to the standard addition method.

<References>

(1) Marreiro et al., "Zinc and Oxidative Stress: Current Mechanisms", *Antioxidants*, 2017
(2) Prabhu et al., "Zinc supplementation decreases galactosylation of recombinant IgG in CHO cells", *Applied Microbiology and Biotechnology*, 2018

Related Products

Application News No. C186A introduced a technique for measuring the organic components in a culture medium. Thus, in addition to the metallic element analysis technique introduced in this article, Shimadzu Corporation can also propose total solutions for culture medium analysis.

By combining these techniques with LC/MS/MS, simultaneous analysis of 125 components, beginning with amino acids in culture media composition and secreted metabolites and also including sugars, vitamins, and organic acids can be completed in under 20 minutes per sample.

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

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