Study on Calibration of Chromlum(Ⅵ) Analyzer

Jianchun Peng[1a], Xianhong Li[1b], Ying Liu[1c], Tingting Ren[1d*], Hongbo Mo[1e]

[1]Chongqing Academy of Metrology and Quality Inspection, Chongqing 401123, China

Abstract—This article presents a novel metrological calibration method for trace chromium(VI) analyzer. The study identifies all factors that affect the accuracy of chromium(VI) analyzers, and proposes calibration items and method for the analyzer based on its working principle. The calibration items include pump set flow error and pump flow stability, set temperature error and temperature control stability of column oven, baseline noise and drift, minimum detection concentration, linear range, and qualitative and quantitative repeatability. Experimental results shows that, the presented method can meet the requirements for the metrological performance evaluation of chromium(VI) analyzer. The calibration method is scientific and feasible.

1. Introduction

Chromium mainly exists in three forms in nature: metallic chromium, chromium(III), and chromium(VI). Chromium(VI) combines with oxyacid, and is widely used in chemical production, metallurgy, electroplating, leather and textile industry, and other fields. Chromium(VI) can enter human body through skin, mucous membrane, respiratory system, etc. causing toxicity and carcinogenicity to multiple organs, including reproductive system, skeletal system, liver, and kidney, The International Cancer Research Structure of the World Health Organization explicitly lists chromium(VI) as a primary carcinogen. Therefore, China has promulgated standard limits and monitoring methods for chromium(VI) in surface water, groundwater, solid waste, food, electronic and electrical products, and other samples. The concentration of chromium(VI) in waste water should be no higher than 0.5 mg/L, and the concentration of chromium(VI) in ambient air should be no higher than 0.00025 mg/m^3. [1-4]

Currently, there are many methods for the measurement of chromium(VI), including 1, 5-diphenylcarbazide photometric method[5], atomic absorption spectrophotometry, chromatographInductively coupled plasma mass spectrometry, ion chromatography with post-column method, etc. Ion chromatography with post-column method is widely recognized as the standard method for the measurement of chromium(VI) in international or national standards, such as ISO 17075-2-2017 *Leather - Chemical determination of chromium(VI) content in leather - Part 2: Chromatographic method* [6], SN/T 2210-2021 *Determination of hexavalent chromium in foods for export* [7], and HJ 779-2015 *Ambient Air - Determination of hexavalent chromium (VI) - Ion Chromatography with post - column method* [8].

Ultra-trace chromium(VI) analyzer is a specialized instrument for measuring chromium(VI) in samples. The sample is separated by chromatography and mixed with a derivative reagent (1, 5-diphenylcarbazide). Using the strong oxidizing property of chromate, 1, 5-diphenylcarbazide is oxidized in an acidic environment and complexed to form a purplish red complex, whose absorbance can be measured at 540 nm by ultra-trace chromium(VI) analyzer. Ultra-trace chromium(VI) analyzer mainly consists of infusion system, sample injection system, separation system, detection system, and data processing system. Currently, there are no calibration specifications or verification regulations for ultra-trace chromium(VI) analyzer in China. This article discusses the calibration method of ultra-trace chromium(VI) analyzer based on the working principle of the analyzer, referring to JJG 705-2014 *Liquid Chromatographs* [9] and JJG 823-2014 *Ion Chromatographs* [10].

2. Calibration standards

2.1. Reference material

The reference material of chromium(VI) in water is used for calibration of minimum detection concentration, linear range, and qualitative and quantitative repeatability, and the relative expanded uncertainty of the reference material should not be greater than 0.2% (*k*=2).

2.2. Standard devices

Analytical balance with maximum weighing capacity of no less than 100 g, and definition of better than 1 mg, as well as stopwatch with definition of better than 0.1 s are used together to calibrated the pump flow set value error and pump flow stability. Pipet and volumetric flask of Class A are used to prepare standard solutions.

[a]755245093@qq.com, [b]38634546@qq.com, [c]718589156@qq.com, [d*]755245093@qq.com, [e]1020875858@qq.com

3. Calibration method and experimental verification

3.1. Calibration of infusion system

3.1.1. Pressure resistance performance of the pump

Connect all parts of the ultra-trace chromium(VI) analyzer, use pure water as the mobile phase, and set the flow rate at 1 mL/min. Start the instrument according to instruction manual. Use filter paper to check if there is leakage at each pipeline interface after the pressure is stable for 10 minutes. Remove the chromatographic column, block the pump outlet (below the pressure sensor), make the pressure reach 90% of the maximum allowable value of the analyzer, and maintain the status for 5 minutes to see if there is leakage.

3.1.2. Pump set flow error and pump flow stability

Start the ultra-trace chromium(VI) analyzer, use pure water as the mobile phase, and set the flow to 0.5 mL/min, 1.0 mL/min, and 2.0 mL/min in turn after the pressure is stabilized. Collect the mobile phase at the outlet of the separation column using volumetric flask

whose weigh W_1 is already know. The time of collecting the mobile phase t is 20 min, 10 min, and 5 min respectively, at every set flow. Weigh the flask with mobile phase in it on the balance, and record the indication of the balance W_2. The measured flow F_m is calculated according to equation (1).

$$F_m = (W_2 - W_1)/(\ell_t t) \qquad (1)$$

Where ℓ_t is density of mobile phase under experimental temperature.

The pump set flow error S_S is calculated according to equation (2).

$$S_S = (\overline{F}_m - F_S)/F_S \times 100\% \qquad (2)$$

Where $\overline{F}_m$ is the average of F_m at the same set flow, and F_s is the set flow.

The pump flow stability S_R is calculated according to equation (3),

$$S_R = (F_{max} - F_{min})/\overline{F}_m \times 100\% \qquad (3)$$

Where F_{max} is the maximum of all measured value of flow under each set flow, while F_{min} is the minimum.

The experimental results of pump set flow error and pump flow stability of a typical type of ultra-trace chromium(VI) analyzer is demonstrated in Table 1.

Table 1 Experimental results of pump set flow error and pump flow stability

Parameter	Value of parameter								
F_S (ml/min)	0.50			1.0			2.0		
t (min)	20			20			5		
W_1 (g)	12.3562	42.2041	72.0532	22.3086	52.1619	82.0097	32.2565	62.1113	91.9565
W_2 (g)	22.3086	52.1619	82.0097	32.2565	62.1113	91.9565	42.2041	72.0532	101.9011
$W_2 - W_1$ (g)	9.9524	9.9578	9.9565	9.9479	9.9494	9.9468	9.9476	9.9419	9.9446
F_m (ml/min)	0.498	0.4988	0.4987	0.9965	0.9967	0.9965	1.9931	1.9920	1.9925
$\overline{F}_m$ (ml/min))	0.4987			0.9966			1.9925		
S_S (%)	-0.26%			-0.34%			-0.37%		
S_R (%)	0.05%			0.03%			0.06%		

As we can see from Table 1, the maximum pump set flow error is -0.37%, and the maximum pump flow stability is 0.06%, both of which meet the requirements of JJG 705-2014 *Liquid Chromatographs* and JJG 823-2014 *Ion Chromatographs*.

3.2. Set temperature error and temperature control stability of column oven

Fix the probe of the digital thermometer as close as possible to the temperature sensor of the column oven or constant temperature block, set the calibration temperature T_S, start the calibrated analyzer, record the

indication of the thermometer every 10 minutes for 7 times after the temperature is stabilized. The set temperature error of column oven ΔT_S is calculated according to equation (4).

$$\Delta T_S = \overline{T} - T_S \qquad (4)$$

Where $\overline{T}$ is the average of all 7 indications of the thermometer.

The temperature control stability of column oven T_C is calculated according to equation (5).

$$T_C = T_{max} - T_{min} \qquad (5)$$

Where T_{max} is the maximum of all 7 indications of the thermometer, while T_{min} is the minimum.

The experimental results of set temperature error and temperature control stability of column oven of the analyzer is demonstrated in Table 2.

Table 2 Experimental results of set temperature error and temperature control stability of column oven

Parameter	Value of parameter						
T_S (℃)				20.0			
Measured temperature (°C)	19.8	19.9	19.8	19.8	19.9	19.8	19.8
$\overline{T}$ (°C)				19.8			
ΔT_S (°C)				-0.2			
T_C (°C)				0.1			
T_S (℃)				40.0			
Measured temperature (°C)	39.8	39.7	39.8	39.8	39.8	39.7	39.8
$\overline{T}$ (°C)				39.8			
ΔT_S (°C)				-0.2			
T_C (°C)				0.1			

As we can see from Table 2, the maximum set temperature error is -0.2 °C, and the maximum temperature control stability of column is 0.1 °C, both of which meet the requirements of JJG 705-2014 *Liquid Chromatographs* and JJG 823-2014 *Ion Chromatographs*.

3.3. Baseline noise and drift

Initialize and preheat the calibrated analyzer, monitor the baseline after the analyzer stabilizes, and record the baseline for 30 minutes. The baseline noise is the signal of the peak height of the peak of maximum noise in the 30 min baseline, and the baseline drift is the maximum signal of the baseline deviation from the starting point within 30 min.

The experimental result of the analyzer is 0.02mAU, and the baseline drift of the analyzer is 0.08mAU/30min, both of which meet the requirements of JJG 705-2014 *Liquid Chromatographs* and JJG 823-2014 *Ion Chromatographs*.

3.4. Minimum detection concentration

Analyze a chromium(VI) standard solution with the concentration of 1.0 ng/mL, inject 100 μL of solution, and the chromatogram is demonstrated in Fig. 1.
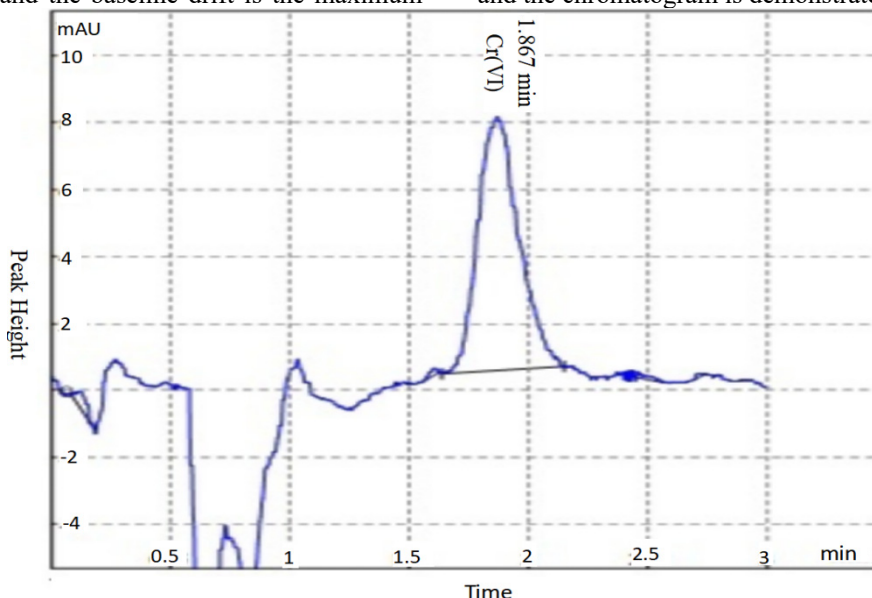

Fig. 1 The chromatogram of chromium(VI) standard solution with the concentration of 1.0 ng/mL

The minimum detection concentration C_{min} is calculated according to equation (6).

$$C_{min} = \frac{2H_N c \times V}{25H} \qquad (6)$$

Where H_N is the peak value of the base line noise peak, c is the concentration of the standard solution, H is the chromatographic peak height the standard solution, and V is the injection volume.

The experimental result of minimum detection concentration of the calibrated analyzer is 0.03 ng/mL,

which meets the requirement of the enterprise standards of manufacturer.

3.5. Linear range

Inject chromium(VI) standard solution with the concentration of 50 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, and 1000 ng/mL in sequence, and the injection amount is 100 µL. Record the response

signal value of each standard solution when it is stable. Repeat the measurement three times at each concentration point to get the average of measured values. Establish a linear regression curve with the measurement results of five concentrations (50ng/mL, 100ng/mL, 200ng/mL, 300ng/mL, and 400ng/mL).

The experimental result of standard curve of the calibrated analyzer is demonstrated in Table 3.

Table 3 Experimental result of standard curve

Concentration (ng/mL)	Response signal value (mAU.s)	Linear regression curve
50	479.58	
100	972.61	
200	1886.83	y=8.9437x+69.869
300	2802.54	
400	3598.63	

We can derive equation (7) from the linear regression curve equation demonstrated in Table 3.

$$x = \frac{y - 69.869}{8.9437} \qquad (7)$$

Where x is the theoretical concentration value of the standard solution calculated by linear regression equation (7), and y is the average of response signal values of the standard solution.

Calculate the linearity error Δx according to equation (8).

$$\Delta x = \frac{x - x_s}{x_s} \times 100\% \qquad (8)$$

Where x_s is the standard concentration of the solution.

The experimental result of linearity error is demonstrated in Table 4.

Table 4 Experimental result of linear error

X_s (ng/mL)	Response signal value (mAU.s)	x	Δx (%)
500	4368.28	488.42	-2.3
600	5385.76	602.18	0.36
700	6045.94	676.00	-3.4
800	6838.44	764.61	-4.4
900	7585.68	848.16	-5.8
1000	8161.72	912.57	-8.7

The upper detection limit C_H is at the minimum concentration whose absolute value of linearity error is more than 5%, and the lower detection limit C_L is the minimum detection concentration obtained from 3.4. The linear range is calculated according to equation (9).

$$Linear\ range = \frac{C_H}{C_L} \qquad (9)$$

As we can see from Table 4, the upper detection limit of the calibrated analyzer is 900 ng/mL, and the minimum detection concentration of the analyzer is 0.03ng/mL, therefore, the linear range of the analyzer is 3×10^4, which meets the requirement of JJG 705-2014 *Liquid Chromatographs*.

Inject 200 µL of standard solution whose concentration is 10 ng/mL, repeat it 7 times, and record the retention time and peak area. Calculate the qualitative and quantitative repeatability *RSD* according to equation (10).

$$RSD = \frac{1}{\bar{x}} \times \sqrt{\frac{\sum_{i=1}^{7} (x_i - \bar{x})^2}{7-1}} \times 100\% \qquad (10)$$

Where x_i is the recorded retention time or peak area, and $\bar{x}$ is the average of retention time or peak area. The experimental results of qualitative and quantitative repeatability is demonstrated in Table 5.

3.6. Qualitative and quantitative repeatability

Table 5 Experimental results of qualitative and quantitative repeatability

	1	2	3	4	5	6	7	Average	Repeatability
Retention time (min)	1.836	1.838	1.835	1.836	1.837	1.837	1.838	1.837	0.06
Peak area (mAU.s)	101.32	100.64	102.11	101.58	103.04	102.61	101.86	101.88	0.79

As we can see from Table 5, the qualitative repeatability is 0.06%, and the quantitative repeatability is 0.79%, both of which meet the requirement of JJG 705-2014 *Liquid Chromatographs*.

4. Conclusion

This article presents metrological calibration method for trace chromium(VI) analyzer based on its working principle. The experimental results show that the calibration method is scientific and practical for metrological performance evaluation of the analyzer, and can be used for daily metrological calibration of it, which can ensure its accuracy and consistency in daily use. This study lays a foundation for the accurate calibration of trace chromium(VI) analyzer. The follow-up study will develop a set of reference materials of chromium(VI) in water whose concentration is more accurately determined, in order to improve the calibration accuracy of the method.

References

1. Xie, W. Q. (2016) Study on the acute and chronic harm of hexavalent chromium to human body. J. Resources Economization & Environment Protection, 7: 131, 135.
2. Liu, Q. X., et al. (2022) Influences and the treatment of hexavalent chromium on male fertility. J. Heilongjiang Journal of Animal Reproduction, 04: 12-17, 23.
3. Chen, L. Q., et al. (2015) Recent advances of researches on determination of hexavalent chromium. J. Physical Testing and Chemical Analysis（Part B:Chemical Analysis）, 08: 1208-1212.
4. Zhu, R. R., et al. (2023) Progress in researches on pulmonary carcinogenicity of hexavalent chromium and its mechanism: a review. J. Chinese Journal of Public Health. 39(2): 181-185.
5. Shang, B. Y., et al. (1987) Water quality - Determination of chromium(VI). 1, 5 Diphenylcarbohydrazide spectrophotometric method. S. China Environmental Press, Beijing.
6. ISO. (2022) ISO 17075-2 Leather - Chemical determination of chromium(VI) content in leather - Part 2: Chromatographic method. S. ISO copyright office, Geneva.
7. Fan, X. (2020) SN/T 2210 Determination of hexavalent chromium in foods for export. S. China Customs Press, Beijing.
8. Ministry of Ecology and Environment of the People's Republic of China. (2015) HJ 779-2015 Ambient Air - Determination of hexavalent chromium (VI) - Ion Chromatography with post - column method. S. China Environmental Press, Beijing.
9. He, Y, J., et al. (2014) JJG 705 Liquid Chromatographs. S. Standards Press of China, Beijing.
10. Shi, N, J., et al. (2014) JJG 823 Ion Chromatographs. S. Standards Press of China, Beijing.