APPLICATION NOTE 73340

Cupric chloride assay by ion chromatography

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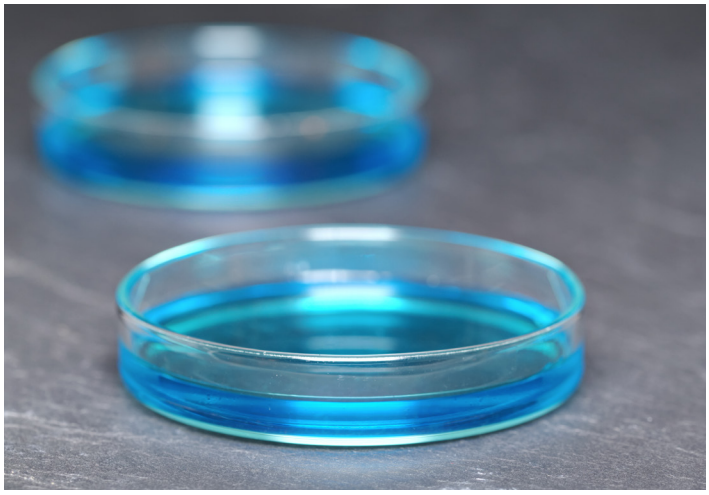

Goals

To develop an assay method for cupric chloride by determining copper using an IC system with absorbance detection

Introduction

Copper is an essential nutrient for humans and thus is an additive to intravenous solutions for total parenteral nutrition to prevent the adverse effects of copper deficiency. Copper is part of several enzymes that regulate a variety of metabolic processes. Even though copper deficiency is rare in humans, it has been reported in patients with prolonged severe malabsorption, premature infants, children recovering from severe malnutrition, and patients receiving parenteral nutrition without copper supplementation.[1] Copper salts such as cupric chloride ($CuCl_2$) are used for nutritional supplementation. The United States Pharmacopeia (USP) monograph for cupric chloride describes an assay for copper that is based on titration.[2] This wet-chemical assay involves first mixing cupric chloride with acetic acid and potassium iodide, followed by titration of liberated iodine with sodium thiosulfate containing potassium thiocyanate. This method is cumbersome and tedious. The USP has initiated an effort to modernize existing monographs across all compendia.[3] In response to this effort, this application note describes an alternative method for copper determination using ion chromatography (IC). The IC assay proposed here is automated, fast, and convenient, offering a significant improvement to the existing assay described in the USP monograph.

This application note describes an IC-based method that uses a Thermo Scientific™ Dionex™ IonPac™ CS5A cation-exchange column, manually prepared pyridine-2,6-dicarboxylic acid (PDCA) eluent, post-column reaction with 4-(2-pyridylazo)resorcinol (PAR), and absorbance detection at 530 nm to determine copper in cupric chloride. The Dionex IonPac CS5A high-resolution column is designed for fast and accurate determination of the transition and lanthanide metals in a variety of samples. This column, in combination with post-column derivatization for visible detection at 530 nm, provides a sensitive and selective method for transition metal analysis. The determination of common transition metals can be achieved in less than 11 min. The method proposed in this application note was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Procedures.[4]

Experimental

Equipment

- A Thermo Scientific™ Dionex™ ICS-5000+ Reagent-Free Ion Chromatography system* including
  - DP Dual Pump with degas option
  - DC Detector Compartment with dual temperature zone
- Thermo Scientific™ Dionex™ AS-AP autosampler (P/N 074926) with tray temperature control option (recommended)
- 6.5 µL sample loop
- 10 mL polypropylene autosampler vials, with caps (P/N 074228)
- Thermo Scientific™ UltiMate™ 3000 series DAD (P/N 5082.0010) and 13 µL analytical PEEK flow cell (P/N 6082.0400)
- Thermo Scientific™ Nalgene™ Rapid-Flow 0.2 µm filter units, 1000 mL, nylon membrane, 90 mm diameter (Fisher Scientific, P/N 164-0020)
- Thermo Scientific™ Dionex™ Knitted Reaction Coil For 2 mm System, 125 µL (P/N 053640) with 3-way manifold (P/N 053593)

* Equivalent results can be achieved using the Thermo Scientific™ Dionex™ ICS-6000 system.

Method conditions	
Columns	• Dionex IonPac CS5A 2 × 250 mm (P/N 052576) • IonPac CG5A Guard 2 × 50 mm (P/N 052836)
Eluent	PDCA (isocratic elution)
Eluent flow rate	0.3 mL/min
Column temperature	30 °C
Post-column reagent (PCR)	PAR
PCR flow rate	0.15 mL/min
PCR reaction coil	125 µL
Detection wavelength	530 nm
Run time	10 min
Injection volume	6.5 µL (Full loop)
Backpressure	~1600 psi

Reagents and chemicals

- Cupric chloride (MilliporeSigma P/N 459097)
- 1000 ppm Copper standard (MilliporeSigma P/N 40786-100ML)
- 1000 ppm Iron standard (MilliporeSigma P/N 149-100ML-F)
- 1000 ppm Nickel standard (MilliporeSigma P/N 42637-100ML)
- Thermo Scientific™ Dionex™ MetPac™ PDCA Eluent Concentrate (P/N 046088)
- Thermo Scientific™ Dionex™ MetPac™ PAR Post Column Diluent (P/N 046094) and Thermo Scientific™ Dionex™ PAR Reagent (P/N 039672)

Preparation of solutions and reagents

It is essential to use high-quality water of high resistivity (18 MΩ•cm) containing as little as possible dissolved carbon dioxide, metal-containing impurities, organics, microorganisms, and particulate matter larger than 0.2 µm. Prior sparging and filtration through a 0.2 µm porosity nylon filter under vacuum is recommended to remove particulates and reduce dissolved air. Keep the eluent solution blanketed under 34–55 kPa (5–8 psi) of helium or nitrogen at all times to reduce contamination from carbon dioxide gas and other contaminants.

- Calibration standards: Calibration standards were prepared by appropriately diluting a 1000 ppm commercial copper standard. Prepare a 100 mg/L secondary stock solution by 10-fold dilution of the primary stock solution in 2 mM HNO_3. Dilute the secondary stock solution appropriately to prepare 10 calibration standards: 10, 7.5, 5, 3, 2, 1, 0.75, 0.5, 0.3, and 0.2 mg/L. Each solution contained 2 mM HNO_3. For example, for a 10 mg/L standard, perform a 1:10 dilution of the 100 mg/L standard by adding 10 g of 100 mg/L standard to a 125 mL polypropylene bottle. Add 0.2 g of 1 M HNO_3. Add DI water to a final weight of 100 g. Cap and store the bottle at 4 °C until needed.
- Copper standards from cupric chloride for recovery studies: A stock solution containing 1000 mg/L copper was prepared using cupric chloride. Accurately weigh 0.268 g of cupric chloride and dissolve in DI water in a 125 mL polypropylene bottle. Add 0.2 g of 1 M HNO_3. Adjust the weight to 100 g with DI water. Cap and store the bottle at 4 °C until needed.

- Iron and nickel standards: Iron and nickel standards were prepared by appropriately diluting 1000 ppm commercial standards. Prepare a 100 mg/L secondary stock solution by 10-fold dilution of the primary stock solution in 2 mM HNO_3. Dilute secondary stock solutions appropriately to prepare solutions containing required iron and nickel concentrations in 2 mM HNO_3, mix and store the bottles at 4 °C until use.

- PDCA eluent solutions: Prepare 1 L of PDCA eluent by diluting 200 mL or 204 g of the Dionex MetPac PDCA Eluent Concentrate with 800 mL of degassed, DI water.

- Post-column PAR reagent solution: Accurately weigh 0.060 g of PAR into 1000 mL of the Dionex MetPac PAR post-column reagent diluent. Sonicate for 10 min to ensure complete dissolution. Protect the reagent from sunlight by using an amber container or covering the reagent bottle with aluminum foil.

System preparation and configuration

To reduce pump fluctuations, install green PEEK (0.030 in. i.d.) tubing (e.g., 10 m) between the eluent pump and injector. In this study, eluent pressure fluctuations were reduced to <0.3% peak-to-peak by performing this step. To minimize absorbance detector noise and drift, use an IC system that has a temperature-controlled compartment to house the column set, mixing tee, and knitted reaction coil. To minimize peak broadening, house the thermostatted items in the same compartment (i.e., the Dionex ICS-5000$^+$ system upper DC compartment). Install the Dionex IonPac CG5A/CS5A column set and condition with the effluent going to waste. Attach the column to the mixing tee, plug one port on the tee, and attach one end of the knitted reaction coil to the third port on the tee. Connect the other end of the knitted reaction coil to the absorbance detector cell inlet. Attach a short piece of red PEEK (0.005 in. i.d.) tubing to the cell output followed by a PEEK union and green PEEK tubing to waste. Turn on the visible lamp in the detector to allow sufficient time (~1 h) for the lamp's output to stabilize. The baseline noise at 530 nm, without introducing PAR post-column reagent, must be 10 µAU or less.

Robustness study

Following the guidelines of USP Physical Tests, <621> Chromatography, method robustness was evaluated by examining retention time (RT), peak asymmetry, and resolution after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature).[5] A standard mixture containing 5 mg/L copper, 0.5 mg/L iron, and 0.5 mg/L nickel was injected in triplicate for each condition. The same procedure was applied to another column set from a different lot.

The variations tested were as follows:

- Flow rate at 0.27 mL/min, 0.3 mL/min, and 0.33 mL/min
- Eluent concentration at 90%, 100%, and 110% PDCA
- Column temperature at 27 °C, 30 °C, and 33 °C

Results and discussion

Separation

Separation of copper was achieved using a Dionex IonPac CS5A, 2 × 250 mm column under isocratic elution conditions. Figure 1 shows a representative chromatogram of a 5 mg/L copper solution using the proposed method. To demonstrate good separation from other transition metal cations that could be in the sample and that are known to elute close to copper, separation of copper from iron and nickel was tested. Figure 2 shows a chromatogram for a sample containing 5 mg/L copper and 0.5 mg/L of iron and nickel separated using the proposed method. Both iron and nickel are well resolved from copper.

Conditions

Column:	Dionex IonPac CG5A Guard, 2 × 50 mm and Dionex IonPac CS5A Analytical, 2 × 250 mm
Eluent:	PDCA
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	6.5 µL (Full Loop)
Detection:	Absorbance at 530 nm after post-column reaction with PAR
Peak:	1. Copper

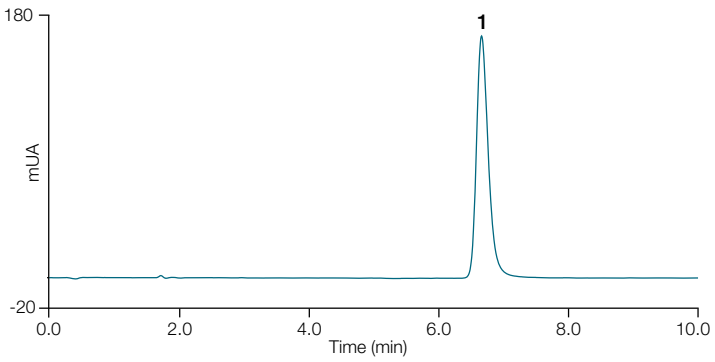

Figure 1. A 5 mg/L solution of copper analyzed on a Dionex IonPac CS5A column

Conditions

Columns:	Dionex IonPac CG5A Guard, 2 × 50 mm and Dionex IonPac CS5A Analytical, 2 × 250 mm
Eluent:	PDCA
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	6.5 µL (Full Loop)
Detection:	Absorbance at 530 nm after post column reaction with PAR
Peaks:	1. Iron 2. Copper 3. Nickel

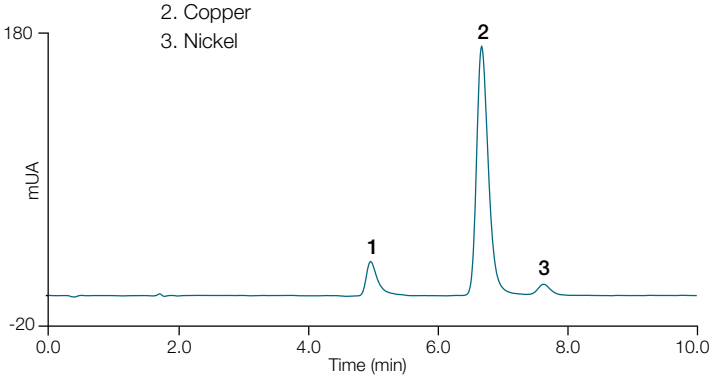

Figure 2. A solution containing 5 mg/L copper, 0.5 mg/L iron, and 0.5 mg/L nickel analyzed on a Dionex IonPac CS5A column

Conditions

Columns:	Dionex IonPac CG5A Guard, 2 × 50 mm and Dionex IonPac CS5A Analytical, 2 × 250 mm
Eluent:	PDCA
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	6.5 µL (Full Loop)
Detection:	Absorbance at 530 nm after post-column reaction with PAR
Peak:	1. Copper

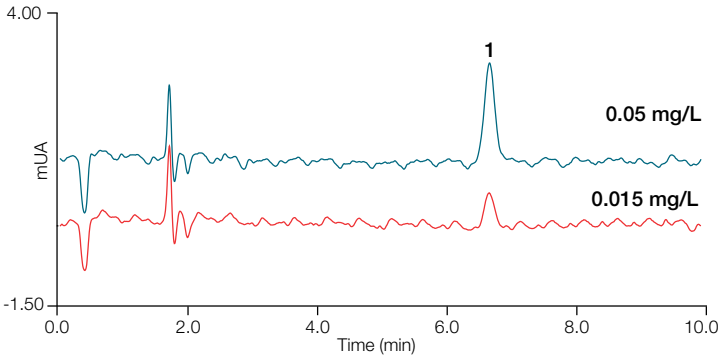

Figure 3. Comparison of 0.05 and 0.015 mg/L solutions of copper

Calibration, LOD, and LOQ

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five standard concentrations to establish linearity in an assay.[5] For a drug substance or finished product, the minimum specified range is from 80% to 120% of the test concentration. A minimum range of 50% to 120% is required for the determination of an impurity. In this study, copper was calibrated at 10 concentration levels ranging from 0.05 to 10 mg/L. A linear relationship of peak area to concentration with a coefficient of determination (r^2) of 0.9999 was obtained (Table 1). To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the copper peak. The signal was determined from the average peak height of seven injections. The LOD and LOQ were then set at concentrations that resulted in signal-to-noise ratios of 3 and 10, respectively (Table 1). Figure 3 shows chromatograms obtained using injections of 0.05 and 0.015 mg/L of copper.

Table 1. Method calibration, LOD, and LOQ data for copper

Parameter	Value	S/N
Linearity (r^2)	0.9999	–
LOD	0.015 mg/L	3.16
LOQ	0.050 mg/L	10.1

Sample analysis

The USP's Cupric Chloride monograph requires that cupric chloride contains not less than 99.0% and not more than 100.5% cupric chloride calculated on the dried basis.[2] In this study, commercially available cupric chloride (99.999%) was used to prepare the test solution of 5 mg/L copper. The calculated concentration of the test solution was 5.03 mg/L, equivalent to 100.23% cupric chloride content (Table 2), thus verifying the label claim. This indicates that the method can determine cupric chloride concentration within the USP specification.

Table 2. Copper recovery studies (n=3)

Base amount (mg/L)	Spiked amount (mg/L)	Spike recovered (mg/L)	Recovery (%)
5	0	5.03	–
	0.5	0.52	103
	1.0	0.99	98.0
	5.0	4.94	98.4

Accuracy and precision

To test sample accuracy, recovery studies were performed after spiking copper samples prepared using a commercially available reference standard. Three different spike levels of 0.05, 1.0, and 5.0 mg/L copper were studied, and satisfactory recoveries were obtained for each spike (Table 2).

The assay precision was evaluated by injecting seven replicates at three different concentration levels of 0.5, 1.0, and 5.0 mg/L copper over five days and expressed as the relative standard deviation (RSD) of retention time (RT) and peak area from the series of measurements. The RT RSDs were ≤0.2% and the peak area RSDs were ≤3.18% (Table 3).

Robustness

The assay robustness was evaluated by measuring the influence of small variations in procedural parameters (e.g., flow rate, eluent concentration, and column temperature) on the RT, peak asymmetry, and resolution from the nickel peak on two columns from different lots. The peak asymmetry was measured using the USP formula.[5] A standard injection (5 mg/L copper, 0.5 mg/L each of iron and nickel) was injected three times (n=3) at each chromatographic condition. Table 4 summarizes the results of the copper robustness study. These results indicate that the method is robust to routine variation in chromatographic parameters and suitable for copper determination.

Table 3. Retention time and peak area precisions of copper solutions (n=7)

Conc. (mg/L)	RSD									
	Day 1		Day 2		Day 3		Day 5		Day 6	
	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area
0.5	0.08	2.97	0.03	2.39	0.04	2.17	0.04	2.08	0.04	1.69
1.0	0.05	3.18	0.05	3.08	0.04	2.49	0.04	1.88	0.20	1.38
5.0	0.09	1.74	0.03	2.26	0.06	1.09	0.02	0.59	0.02	0.82

Table 4. Robustness of the IC-based assay for copper determination performed using a sample containing 5 mg/L copper, 0.5 mg/L each of iron and nickel (n=3)

Method condition	Difference from the standard condition (%)					
	Column 1			Column 2		
	RT	Asymmetry	Resolution (to Ni)	RT	Asymmetry	Resolution (to Ni)
+10% Flow	10.70	2.02	4.97	10.79	–0.32	1.00
–10% Flow	–8.89	–3.02	–3.20	–8.97	0.32	–0.68
–10% PCR Flow	0.25	0.50	2.01	0.19	0.19	–0.10
+10% PCR Flow	0.34	–1.26	1.89	–0.35	0.00	–0.03
+10% Temperature	5.48	–0.25	0.83	5.48	0.00	–0.04
–10% Temperature	–5.02	–2.27	0.95	–5.22	0.39	0.68
+10% Eluent	1.68	–0.25	3.55	4.01	0.06	1.91
–10% Eluent	0.52	–1.01	1.54	–3.88	0.58	–1.59

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Conclusion

This study describes an IC-based assay for cupric chloride. Copper was separated on a cation-exchange column and detected by absorbance at 530 nm. This method allows the concentration of copper to be determined in an automated way rather than performing the cumbersome titration-based assay. This assay for copper chloride was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures, and was shown to measure the copper content of cupric chloride accurately. Compared to the assay in the official USP Cupric Chloride monograph, this IC-based assay offers a simple, accurate, and robust measurement without excessive handling of hazardous reagents. Therefore, this method is a candidate to replace the existing assay for copper in the USP monograph, and thereby modernize the monograph.

References

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