Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-May-2022
Document Type: General Chapter
DocId: GUID-E32AF3DF-5989-473B-A52A-99000BF0DBDB_5_en-US
DOI: https://doi.org/10.31003/USPNF_M99350_05_01
DOI Ref: 6rd5b

⟨591⟩ ZINC DETERMINATION

INTRODUCTION

A quantitative determination of zinc in drug substance and drug product monographs containing zinc is achieved by the *Dithizone Method*, *Ion Chromatographic Method*, or *Atomic Absorption Method*. Unless a specific method is indicated in the individual monograph, any of these methods can be used.

PROCEDURE

• Dithizone Method

Select all reagents for this test to have as low a content of heavy metals as practicable. If necessary, distill water and other solvents into a hard or borosilicate glass apparatus. Rinse thoroughly all glassware with warm diluted nitric acid (1 in 2) followed by water. Avoid using on the separator any lubricants that dissolve in chloroform.

Special solutions and solvents

Alkaline ammonium citrate solution: Dissolve 50 g of dibasic ammonium citrate in water to make 100 mL. Add 100 mL of ammonium hydroxide. Remove any heavy metals that may be present by extracting the solution with 20-mL portions of the *Dithizone extraction solution* (see *Lead* ⟨251⟩) until the *Dithizone extraction solution* retains a clear green color, then extract any dithizone remaining in the citrate solution by shaking with chloroform.

Chloroform: Distill chloroform in a hard or borosilicate glass apparatus, receiving the distillate in sufficient dehydrated alcohol to make the final concentration 1 mL of alcohol for each 100 mL of distillate.

Dithizone solution: Use the *Standard dithizone solution* (see ⟨251⟩), prepared with the distilled *Chloroform*.

Standard zinc solution: Dissolve 625 mg of zinc oxide, accurately weighed and previously gently ignited to constant weight, in 10 mL of nitric acid, and add water to make 500.0 mL. This solution contains 1.0 mg/mL of zinc.

Diluted standard zinc solution: Dilute 1 mL of the *Standard zinc solution*, accurately measured, with 2 drops of nitric acid and sufficient water to make 100.0 mL. This solution contains 10 µg/mL of zinc. Use this solution within 2 weeks.

Trichloroacetic acid solution: Dissolve 100 g of trichloroacetic acid in water to make 1000 mL.

Procedure: Transfer 1–5 mL of the preparation to be tested, accurately measured, to a centrifuge tube graduated at 40 mL. If necessary, add 0.25 N hydrochloric acid, dropwise, to obtain a clear solution. Add 5 mL of the *Trichloroacetic acid solution* and sufficient water to make 40.0 mL. Mix and centrifuge.

Transfer to a hard-glass separator an accurately measured volume of the supernatant believed to contain 5–20 µg of zinc, and add water to make about 20 mL. Add 1.5 mL of the *Alkaline ammonium citrate solution* and 35 mL of the *Dithizone solution*. Shake vigorously 100 times. Allow the chloroform phase to separate. Insert a cotton plug in the stem of the separator to remove any water emulsified with the chloroform. Collect the chloroform extract (discarding the first portion that comes through) in a test tube, and determine the absorbance at 530 nm, with a suitable spectrophotometer.

Calculate the amount of zinc present by reference to a standard absorbance–concentration curve obtained by using 0.5, 1.0, 1.5 mL, and, if the zinc content of the sample extracted exceeds 15 µg, 2.0 mL of the *Diluted standard zinc solution*, corrected as indicated by a blank determination run concomitantly, using all of the reagents but no added zinc.

• Ion Chromatographic Method

The following ion chromatographic general procedure is provided for the determination of zinc in compendial articles. See *Ion Chromatography* ⟨1065⟩ for discussion of the theory and principles of measurements using ion chromatography.

Use water with a resistivity of not less than 18 megohm-cm to prepare the solutions.

Diluent: 0.2% (w/v) hydrochloric acid

Mobile phase: 7.0 mM dipicolinic acid, 66.0 mM potassium hydroxide, 5.6 mM potassium sulfate, and 74.0 mM formic acid in water; adjust with 2 N potassium hydroxide TS to a pH of 4.2. Pass through a suitable filter of 0.2-µm pore size.

Post-column derivatization reagent: 0.5 mM 4-(2-pyridylazo)resorcinol monosodium salt, 1.0 M 2-dimethylaminoethanol, 0.50 M ammonium hydroxide, and 0.30 M sodium bicarbonate in water. Stir and sonicate until the solid is completely dissolved. Pass through a suitable filter of 0.2-µm pore size.

Standard stock solution: 1500 µg/mL of zinc from USP Zinc Oxide RS prepared as follows. Transfer an appropriate portion of USP Zinc Oxide RS to a suitable volumetric flask. Add 6 N hydrochloric acid to about 10% of the final flask volume to dissolve. Dilute with water to volume.

Standard solution: 15.0 µg/mL of zinc in *Diluent* from the *Standard stock solution*

Sample stock solution: Prepare as directed in the monograph.

Sample solution: Equivalent to 15.0 µg/mL of zinc in *Diluent* from the *Sample stock solution*, unless otherwise stated in the monograph

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: Vis 530 nm

Columns

Guard: 4.0-mm × 5-cm; 9-µm packing L100

Analytical: 4.0-mm × 25-cm; 9-µm packing L100

Column temperature: 30°

Flow rate: 1.2 mL/min

Flow rate of post-column reagent: 0.6 mL/min. Introduce using a pulseless flow of reagent through a 375-µL polymeric mixing coil or other suitable volume coil.[1]

Injection volume: 10 µL

Run time: Not less than 2 times the retention time of zinc

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: Not more than 2.0

Relative standard deviation: Not more than 0.73%, unless otherwise stated in the monograph

Analysis

Samples: *Standard solution* and *Sample solution*

Unless otherwise stated in the monograph, calculate the concentration of zinc in the portion of *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of zinc in the *Standard solution* (µg/mL)

Change to read:

• **Atomic Absorption Method**

The following atomic absorption general procedure is provided for the determination of zinc in compendial articles.

Diluent: 0.01 N hydrochloric acid

Zinc stock standard solution: Use a certified 1000-µg/mL standard.

▲▲ (USP 1-May-2022)

Zinc calibration standard solutions: Prepare not less than 3 calibration standard solutions within the range of 0.2–1.6 µg/mL, or suitable concentrations to span anticipated zinc concentrations in the sample(s). Prepare these solutions by two-step dilutions of the *Zinc stock standard solution* with the *Diluent*.

Drug substance sample solution: Accurately weigh an amount of the sample and dissolve in *Diluent*. Dilute with *Diluent* to volume in a volumetric flask; dilute the solution such that the zinc concentration is within the concentration range of the prepared *Zinc calibration standard solutions*.

Drug product sample solution: If the sample is a suspension, resuspend and add 4–7 µL of 6 N hydrochloric acid,▲to each milliliter of the suspension▲ (USP 1-May-2022) as needed to dissolve prior to dilution. Dilute the sample with *Diluent* to a zinc concentration within the concentration range of the prepared *Zinc calibration standard solutions*.

Instrumental conditions

(See *Atomic Absorption Spectroscopy ⟨852⟩*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 213.9 nm

Lamp: Zinc hollow-cathode, 5–15 mA

Flame: Air–acetylene of suitable composition

Blank: 0.01 N hydrochloric acid

Analysis: Zero the instrument using the *Blank*. Determine the absorbance in triplicate, of the *Blank*, ▲▲ (USP 1-May-2022) the *Zinc calibration standard solutions*, and the *Sample solutions*.

Calibration: Prepare a calibration curve from the mean of the readings of the absorbance of the *Blank* and the *Zinc calibration standard solutions* (linear or quadratic fit).

System suitability

▲**Samples:** *Zinc calibration standard solutions*

Suitability requirements

Correlation coefficient: NLT 0.997, determined from the standard curve

Relative standard deviation: NMT 2.5%, for the triplicate measurements for each aspiration of the *Zinc calibration standard solutions* with the highest concentration▲ (USP 1-May-2022)

Calculations: Read the concentration of zinc in the sample solution using the calibration curve and calculate the concentration of zinc in the sample.

ADDITIONAL REQUIREMENTS

- **USP Reference Standards ⟨11⟩**
  USP Zinc Oxide RS

[1] A knitted reaction coil, part number 043700, available from ThermoFisher Scientific (www.thermofisher.com), may be suitable.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<591> ZINC DETERMINATION	Edmond Biba Senior Scientific Liaison	GCCA2020 General Chapters - Chemical Analysis 2020

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 46(6)

Current DocID: GUID-E32AF3DF-5989-473B-A52A-99000BF0DBDB_5_en-US
DOI: https://doi.org/10.31003/USPNF_M99350_05_01
DOI ref: 6rd5b