in *Barium acetate buffer* for 10 min, or until evenly wetted, then blot dry between two sheets of absorbent paper. Using an applicator[2] suitable for electrophoresis, apply equal volumes (0.5 µL) of the *Sample solution* and *Standard solution* to the brighter side of the membrane held in position in an appropriate applicator stand or on a separating bridge in the chamber. Ensure that both ends of the membrane are dipped at least 0.5–1.0-cm deep into the buffer chambers. Apply a constant 60 volts (6 mA at the start) for 2 h. [NOTE—Perform the application of solutions and voltage within 5 min because further drying of the blotted paper reduces sensitivity.]

Place the membrane in a plastic staining tray, and with the application side down, float or gently immerse in *Staining reagent* for 5 min. Then stir the solution gently for 1 min. Remove the membrane, and destain in 5% acetic acid until the background clears.

**Acceptance criteria:** The principal spot from the *Sample solution* has the same migration as the principal spot from the *Standard solution*. [NOTE—Document the results by taking a picture within 15 min of completion of destaining.]

## STRENGTH

- **CONTENT OF CHONDROITIN SULFATE SODIUM**

**Standard solutions:** 1.5, 1.0, and 0.5 mg/mL of USP Chondroitin Sulfate Sodium RS in water

**Sample solution:** Transfer an equivalent to 100 mg of chondroitin sulfate sodium from NLT 20 Tablets, finely powdered, to 60 mL of water, and shake to suspend the powder in solution. Sonicate in a 65° water bath for 20 min. Remove from the bath, stir or shake for 5 min, dilute with water to 100 mL, and centrifuge or pass through a suitable filter.

**Diluent:** Weigh about 297 mg of monobasic potassium phosphate, 492 mg of dibasic potassium phosphate, and 250 mg of polysorbate 80, and transfer into a 1-L beaker. Dissolve in approximately 900 mL of water, and adjust with potassium hydroxide or phosphoric acid to a pH of 7.0 ± 0.2. Dilute with water to 1 L, and mix thoroughly.

**Titrimetric system**

(See *Titrimetry* ⟨541⟩.)

**Mode:** Photometric titration

**Titrant:** 1 mg/mL of cetylpyridinium chloride in water

**Endpoint detection:** Turbidimetric with photoelectric probe

**Analysis:** Transfer 5.0 mL of each *Standard solution* and the *Sample solution* to separate titration vessels, and add 25 mL of *Diluent* to each. Stir until a steady reading is obtained with a photoelectric probe either at 420, 550, or 660 nm. Set the instrument to zero in absorbance mode. Titrate with *Titrant* using the photoelectric probe to determine the endpoint turbidimetrically. From a linear regression equation, calculated using the volumes of *Titrant* consumed versus concentrations of the *Standard solutions*, determine the concentration of chondroitin sulfate sodium in the *Sample solution*.

Calculate the percentage of the labeled amount of chondroitin sulfate sodium in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times 100$$

$C$ = determined concentration of chondroitin sulfate sodium in the *Sample solution* (mg/mL)

$C_U$ = nominal concentration of chondroitin sulfate sodium in the *Sample solution* (mg/mL)

[2] Suitable applicators are available from DiaSys Corp., Waterbury, CT (www.diasys.com) and Helena Laboratories, Beaumont, TX (www.helena.com).

**Acceptance criteria:** 90.0%–120.0% of the label claim

## PERFORMANCE TESTS

- **DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS ⟨2040⟩:** Meet the requirements for *Dissolution*

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Titrant** and **Diluent:** Prepare as directed as in *Content of Chondroitin Sulfate Sodium*.

**Standard solutions:** 1.5 , 1.0, and 0.5 mg/mL of USP Chondroitin Sulfate Sodium RS in water

**Sample solution:** Combine equal portions of the solutions withdrawn from 6 dissolution vessels and pass through a suitable filter; use the pooled sample as the test specimen.

**Analysis:** Transfer 5.0 mL of each *Standard solution*, and an aliquot of the *Sample solution* equivalent to about 5 mg of chondroitin sulfate sodium, to separate titration vessels. Add 25 mL of *Diluent* to each titration vessel. Stir until a steady reading is obtained with a photoelectric probe. Set the instrument to zero in absorbance mode. Titrate with *Titrant* using the photoelectric probe to determine the endpoint turbidimetrically, either at 420, 550, or 660 nm. From a linear regression equation, calculated using the volumes of *Titrant* consumed versus amount, in mg, of chondroitin sulfate sodium from each *Standard solution*, determine the amount, in mg, of chondroitin sulfate sodium in the aliquot of *Sample solution* taken.

Calculate the percentage of the labeled amount of chondroitin sulfate sodium dissolved:

$$\text{Result} = (Ws/a) \times (V/L) \times 100$$

$Ws$ = amount of chondroitin sulfate sodium in the aliquot of the *Sample solution* taken (mg)

$a$ = volume of the aliquot of *Sample solution* taken

$V$ = volume of *Medium*, 900 mL

$L$ = label claim of chondroitin sulfate sodium (mg/Tablet)

**Tolerances:** NLT 75% of the labeled amount of chondroitin sulfate sodium is dissolved.

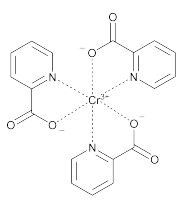
- **WEIGHT VARIATION OF DIETARY SUPPLEMENTS ⟨2091⟩:** Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **LABELING:** Label it to indicate the species of the source from which the chondroitin used to prepare the Tablets was derived. Label it to state the source(s) of chondroitin sulfate sodium, whether bovine, porcine, avian, or a mixture of any of them. The label states on the front panel the content of chondroitin sulfate sodium on the dried basis.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Chondroitin Sulfate Sodium RS

# Chromium Picolinate



$C_{18}H_{12}N_3O_6Cr$ 418.31

Chromium Tripicolinate [14639-25-9].

**DEFINITION**
Chromium Picolinate contains NLT 98.0% and NMT 102.0% of chromium picolinate ($C_{18}H_{12}N_3O_6Cr$), calculated on the dried basis.

**IDENTIFICATION**
- **A. Infrared Absorption** ⟨**197M**⟩
- **B.**

**Sample solution:** 4 mg/mL
**Analysis:** To 5 mL of the *Sample solution* add 1 mL of 5 N sodium hydroxide and 10 drops of 30% hydrogen peroxide, and heat gently for 2 min.
**Acceptance criteria:** A yellow color develops.

**ASSAY**
- **Procedure**

**Standard stock solution:** 100 µg/mL of chromium. Transfer 0.283 g of potassium dichromate, previously dried at 120° for 4 h, to a 1000-mL volumetric flask, and dilute with water to volume. Store in a polyethylene bottle.
**Standard solutions:** 1.0, 2.0, 3.0, and 4.0 µg/mL of chromium. Separately transfer 1.0 and 2.0 mL of the *Standard stock solution* to 100-mL volumetric flasks, and transfer 1.5 and 2.0 mL of the *Standard stock solution* to separate 50-mL volumetric flasks. Add 1.0 mL of nitric acid to each flask, and dilute the contents of each flask with water to volume.
**Sample solution:** Transfer 200 mg of Chromium Picolinate to a 100-mL beaker, and add 25 mL of water. Slowly add 10 mL of nitric acid, and boil for 10 min with constant swirling. Cool the solution, quantitatively transfer to a 500-mL volumetric flask, and dilute with water to volume. Filter a portion of the solution, and transfer 5.0 mL of the filtrate to a 100-mL volumetric flask. Add 1 mL of nitric acid, and dilute with water to volume.
**Instrumental conditions**
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
**Mode:** Atomic absorption spectrophotometry
**Analytical wavelength:** 357.9 nm
**Lamp:** Chromium hollow-cathode
**Flame:** Air–acetylene
**Blank:** Diluted nitric acid
**Analysis**
**Samples:** *Standard solutions* and *Sample solution*
Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the chromium concentration, in µg/mL, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the chromium concentration, in µg/mL, in the *Sample solution*.
Calculate the percentage of chromium picolinate ($C_{18}H_{12}N_3O_6Cr$) in the portion of Chromium Picolinate taken:

$$\text{Result} = (C_{Cr}/C_U) \times (M_r/A_r) \times 100$$

$C_{Cr}$ = concentration of chromium in the *Sample solution*, obtained from the graph (µg/mL)
$C_U$ = concentration of Chromium Picolinate in the *Sample solution* (µg/mL)
$M_r$ = molecular weight of chromium picolinate, 418.31
$A_r$ = atomic weight of chromium, 51.996

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**
- **Chloride and Sulfate,** *Chloride* ⟨**221**⟩

**Sample solution:** Dissolve 30 mg of Chromium Picolinate in 30–40 mL of water, and heat to 70°. Cool overnight, and filter to remove the precipitate.
**Analysis:** Add 1 mL each of nitric acid and silver nitrate TS, and add sufficient water to make 50 mL. Mix, and allow to stand for 5 min, protected from direct sunlight.
**Acceptance criteria:** Any turbidity formed is NMT that produced in a similarly treated control solution containing 0.25 mL of 0.002 N hydrochloric acid (NMT 0.06%).
- **Chloride and Sulfate,** *Sulfate* ⟨**221**⟩

**Sample solution:** Dissolve 100 mg of Chromium Picolinate in 30–40 mL of water, and heat to 90°. Cool overnight, and filter to remove the precipitate.
**Analysis:** Add 1 mL of 3 N hydrochloric acid, 3 mL of barium chloride TS, and sufficient water to make 50 mL. Mix, and allow to stand for 10 min.
**Acceptance criteria:** Any turbidity formed is NMT that produced in a similarly treated control solution containing 0.2 mL of 0.02 N sulfuric acid (NMT 0.2%).

**SPECIFIC TESTS**
- **Loss on Drying** ⟨**731**⟩**:** Dry a sample at 105° for 4 h: it loses NMT 4.0% of its weight.

**ADDITIONAL REQUIREMENTS**
- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨**11**⟩
  USP Chromium Picolinate RS

## Chromium Picolinate Tablets

**DEFINITION**
Chromium Picolinate Tablets contain NLT 95.0% and NMT 125.0% of the labeled amount of chromium (Cr).

**IDENTIFICATION**
- **A.** The *Sample solution* prepared as directed in the test for *Strength* gives a positive test for chromium, determined at 357.9 nm using the *Instrumental conditions* in the test for *Content of Chromium*.

**STRENGTH**
- **Content of Chromium**

**Standard stock solution A:** 1000 µg/mL of chromium from potassium dichromate, previously dried at 120° for 4 h, in water. Store in a polyethylene bottle.
**Standard stock solution B:** Transfer 1.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, add 5.0 mL of 6 N hydrochloric acid, and dilute with water to volume to obtain a solution having a concentration of 10 µg/mL of chromium.
**Standard solutions:** Dilute *Standard stock solution B* with 0.125 N hydrochloric acid to obtain concentrations of 1.0, 2.0, 3.0, and 4.0 µg/mL of chromium.
**Sample solution:** Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 5 Tablets, to a porcelain crucible, heat the crucible in a muffle furnace maintained at about 550° for 6–12 h, and cool. Add 60 mL of hydrochloric acid, and boil gently on a hot plate or steam bath for 30 min, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and transfer the contents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute this solution with 0.125 N hydrochloric acid to obtain a solution having a concentration of 2.5 µg/mL of chromium.
**Instrumental conditions**
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
**Mode:** Atomic absorption spectrophotometry
**Lamp:** Chromium hollow-cathode
**Flame:** Air–acetylene
**Analytical wavelength:** 357.9 nm (chromium emission line)