$S$ = slope of the *Standard response line* for the electrode

**Acceptance criteria:** NMT 10 µg/g

- **CHROMATOGRAPHIC PURITY**

**Buffer solution:** 7.1 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** *Buffer solution* and acetonitrile (7:3)

**Standard solution:** Transfer an amount, NMT 100 mg, of USP Choline Chloride RS to a 24-mL screw-capped vial, and add 400 mg of 3,5-dinitrobenzoyl chloride and 10 mL of acetonitrile. Cap the vial, heat to 55°, and continue heating for 2 h. Cool to room temperature, and add 5 mL of water. Allow to stand for 5 min. Quantitatively transfer the solution to a 25-mL volumetric flask and dilute with acetonitrile to volume. Dilute a volume of this solution with *Mobile phase* to obtain a concentration of 2.0 µg/mL of USP Choline Chloride RS.

**Sample solution:** Transfer 500 mg of Choline Bitartrate to a centrifuge tube, add 2.0 mL of water, and swirl to dissolve. Add 0.5 mL of potassium chloride solution (7.5 in 25), centrifuge, and transfer 1.0 mL of the supernatant to a 24-mL screw-capped vial. Dry at 120° for 2 h. Add 400 mg of 3,5-dinitrobenzoyl chloride and 10 mL of acetonitrile. Cap the vial, and heat at 55° for 2 h. Cool to room temperature, add 5 mL of water, and allow to stand for 5 min. Quantitatively transfer this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Pipet 2.0 mL of the solution to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 208 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Capacity factor (k′):** NLT 2

**Relative standard deviation:** NMT 5%, determined from the choline derivative peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Choline Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$ = peak response for each impurity, excluding that for the choline derivative and 3,5-dinitrobenzoic acid from the *Sample solution*

$r_S$ = peak response for the choline derivative from the *Standard solution*

$C_S$ = concentration of USP Choline Chloride RS in the *Standard solution* (mg/mL)

$C_U$ = concentration of Choline Bitartrate in the *Sample solution* (mg/mL)

$M_{r1}$ = molecular weight of choline bitartrate, 253.25

$M_{r2}$ = molecular weight of choline chloride, 139.62

**Acceptance criteria**

**Individual impurities:** NMT 0.3%

**Total impurity:** NMT 2.0%

**SPECIFIC TESTS**

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩

**Sample solution:** 400 mg/mL in water

**Acceptance criteria:** +17.5° to +18.5°

- **pH** ⟨**791**⟩**:** 3.0–4.0, in a solution (1 in 10)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 0.5%

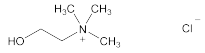
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Choline Bitartrate RS

USP Choline Chloride RS

## Choline Chloride



$C_5H_{14}ClNO$ 139.62

(2-Hydroxyethyl)trimethylammonium chloride;
2-Hydroxy-*N,N,N*-trimethylethanaminium chloride [67-48-1].

**DEFINITION**

Choline Chloride contains NLT 99.0% and NMT 100.5% of choline chloride ($C_5H_{14}ClNO$), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** ⟨**197K**⟩
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨**191**⟩**:** A solution (1 in 20) meets the requirements.

**ASSAY**

- **PROCEDURE**

**Sample:** 120 mg

**Titrimetric system**

(See *Titrimetry* ⟨541⟩.)

**Mode:** Direct titration

**Titrant:** 0.1 N silver nitrate VS

**Endpoint detection:** Potentiometric

**Blank:** 35 mL of water. Add 3 drops of acetic acid.

**Analysis:** Dissolve the *Sample* in 35 mL of water and add 3 drops of acetic acid. Titrate with *Titrant*.

Calculate the percentage of choline chloride ($C_5H_{14}ClNO$) in the *Sample* taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

$V$ = *Sample* titrant volume (mL)

$B$ = *Blank* titrant volume (mL)

$N$ = titrant normality (mEq/mL)

$F$ = equivalency factor, 139.6 mg/mEq

$W$ = weight of the *Sample* (mg)

**Acceptance criteria:** 99.0%–100.5% on the anhydrous basis

**IMPURITIES**

- **RESIDUAL SOLVENTS** ⟨**467**⟩**:** Meets the requirements, except that the limit for 1,4-dioxane is 10 µg/g
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.05%
- **ARSENIC,** *Method I* ⟨**211**⟩

**Analysis:** Add 30 mL of water and 5 mL of hydrochloric acid to dissolve the sample.

DS Monographs

**Acceptance criteria:** NMT 2 ppm

- **LEAD ⟨251⟩**

[NOTE—Use methylene chloride in place of chloroform to prepare the *Dithizone Extraction Solution* and *Standard Dithizone Solution*.]

**Solution A:** Transfer 8.4 g of sodium hydroxide solution (1 in 2) to a plastic bottle, add 100 mL of ammonium hydroxide, and mix.

**Standard solution:** Transfer 1.0 mL of the *Diluted Standard Lead Solution* to a separatory funnel containing 25.0 mL of water.

**Sample solution:** Dissolve 3.00 g of Choline Chloride in a separatory funnel containing 25.0 mL of water.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Separately add 6.0 mL of *Ammonium Citrate Solution* and 3.0 mL of *Potassium Cyanide Solution* to the *Standard solution* and the *Sample solution*. Extract each of the resulting solutions three times with 5.0-mL portions of *Dithizone Extraction Solution*, shaking for 60 s and draining off each extract into another separator. Shake the combined dithizone solutions for 30 s with 20.0 mL of nitric acid (1 in 100), and discard the methylene chloride layer. Add 6.0 mL of *Ammonia–Cyanide Solution*, 2 mL of *Solution A*, and 10 mL of *Standard Dithizone Solution*, and shake for 45 s. Allow the phases to separate, and measure the absorbance of the lower layer at 510 nm with a suitable spectrophotometer.

**Acceptance criteria:** The absorbance of the *Sample solution* is NMT the absorbance of the *Standard solution* (NMT 0.3 ppm).

***Delete the following:***

•• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm• (Official 1-Jan-2018)

- **LIMIT OF TOTAL AMINES**

**Standard solution:** 500 µg/mL of trimethylamine hydrochloride in water

**Sample solution:** Transfer 10.0 g of Choline Chloride to a beaker containing a plastic-coated stirring bar, add 170 mL of water and 30.0 mL of sodium hydroxide TS, and stir until dissolved.

**System suitability stock solution:** 10 µg/mL of trimethylamine hydrochloride in water

**System suitability solution:** Transfer 10.0 mL of *System suitability stock solution* to a beaker containing a plastic-coated stirring bar, add 160 mL of water and 30.0 mL of sodium hydroxide TS, and stir until dissolved.

**Electrode system:** Use a gas-sensing, ammonia-specific indicating electrode with internal reference connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.1 mV (see *pH* ⟨791⟩).

**Standard response line:** Mix 30.0 mL of sodium hydroxide TS, and 170 mL of water. Add a plastic-coated stirring bar, insert the electrode into the solution, and record the potential, in mV. Continue stirring, and at 5-min intervals add 0.200, 0.600, 1.00, and 2.00 mL of *Standard solution*, and record the potential after each addition. Plot the logarithms of the cumulative trimethylamine hydrochloride concentrations (0.50, 1.50, 2.50, and 5.00 µg/mL) versus potential, in mV, and determine the slope (*S*) of the *Standard response line* for the electrode.

**System suitability**

**Sample:** *System suitability solution*

Proceed as directed in *Analysis*, except to replace the *Sample solution* with the *System suitability solution* and in the formula below to replace *W* with *V*, which equals 10 mL.

**Suitability requirements:** The total change is NLT 10 mV for a 0.4-mL cumulative addition of the *Standard solution*; the amount of trimethylamine hydrochloride found is 8.5–11.5 µg/L.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Rinse the electrode, insert it into the *Sample solution*, stir, and record the potential, in mV. Add 0.100 mL of the *Standard solution*, and record the potential. Add another 0.100 mL of the *Standard solution*, and record the potential. [NOTE—If the total change after the second addition of the *Standard solution* is less than 10 mV, add a third aliquot of 0.200 mL.]

Calculate the content, in µg/g, of total amines as trimethylamine hydrochloride in the portion of sample taken:

$$\text{Result} = (C_S \times V_A)/[(F - 1) \times W]$$

$C_S$ = concentration of *Standard solution* (µg/mL)
$V_A$ = total volume of the *Standard solution* added to the *Sample solution* (mL)
$W$ = weight of Choline Chloride taken to prepare the *Sample solution* (g)
$F$ = correction factor, calculated by the formula:

$$F = \text{antilog}\,[(mV_F - mV_0)/S]$$

$mV_F$ = final reading after the additions of the *Standard solution* (mV)
$mV_0$ = initial reading of the *Sample solution* (mV)
$S$ = slope of the *Standard response line* for the electrode

**Acceptance criteria:** NMT 10 µg/g

- **CHROMATOGRAPHIC PURITY**

**Buffer solution:** 7.1 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** *Buffer solution* and acetonitrile (7:3)

**Standard solution:** Transfer an amount, NMT 100 mg, of USP Choline Chloride RS to a 24-mL screw-capped vial, and add 400 mg of 3,5-dinitrobenzoyl chloride and 10 mL of acetonitrile. Cap the vial, heat to 55°, and continue heating for 2 h. Cool to room temperature, and add 5 mL of water. Allow to stand for 5 min. Quantitatively transfer the solution to a 25-mL volumetric flask, and dilute with acetonitrile to volume. Dilute a volume of this solution with *Mobile phase* to obtain a concentration of 2.0 µg/mL of USP Choline Chloride RS.

**Sample solution:** Transfer 110 mg of Choline Chloride to a 24-mL screw-capped vial. Dry at 120° for 2 h. Add 400 mg of 3,5-dinitrobenzoyl chloride and 10 mL of acetonitrile. Cap the vial, heat to 55°, and continue heating for 2 h. Cool to room temperature, and add 5 mL of water. Allow to stand for 5 min. Quantitatively transfer the solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Pipet 2.0 mL of the solution to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 208 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Capacity factor (k′):** NLT 2

**Relative standard deviation:** NMT 5%, determined from the choline derivative peak

DS Monographs

**Analysis**
**Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Choline Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$ = peak response for each impurity, excluding that for the choline derivative and 3,5-dinitrobenzoic acid from the *Sample solution*
$r_S$ = peak response for the choline derivative from the *Standard solution*
$C_S$ = concentration of USP Choline Chloride RS in the *Standard solution* (mg/mL)
$C_U$ = concentration of Choline Chloride in the *Sample solution* (mg/mL)

**Acceptance criteria**
**Individual impurities:** NMT 0.3%
**Total impurities:** NMT 2.0%

**SPECIFIC TESTS**
- **pH** ⟨**791**⟩**:** 4.0–7.0, in a solution (1 in 10)
- **Water Determination,** *Method I* ⟨**921**⟩**:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨**11**⟩
USP Choline Chloride RS

## Chondroitin Sulfate Sodium

Chondroitin, hydrogen sulfate, sodium salt [9082-07-9].

**DEFINITION**
Chondroitin Sulfate Sodium is the sodium salt of the sulfated linear glycosaminoglycan obtained from bovine, porcine, or avian cartilages of healthy and domestic animals used for food by humans. Chondroitin Sulfate Sodium consists mostly of the sodium salt of the sulfate ester of *N*-acetylchondrosamine (2-acetamido-2-deoxy-$\beta$-D-galactopyranose) and D-glucuronic acid copolymer. These hexoses are alternately linked $\beta$-1,4 and $\beta$-1,3 in the polymer. Chondrosamine moieties in the prevalent glycosaminoglycan are monosulfated primarily on position 4 and less so on position 6. It contains NLT 90.0% and NMT 105.0% of chondroitin sulfate sodium, calculated on the dried basis.
[NOTE—Chondroitin Sulfate Sodium is extremely hygroscopic once dried. Avoid exposure to the atmosphere, and weigh promptly.]

**IDENTIFICATION**
- **A. Infrared Absorption** ⟨**197K**⟩
- **B. Identification Tests—General** ⟨**191**⟩**,** *Sodium*
**Sample solution:** 0.5 g in 10 mL of water
**Acceptance criteria:** Meets the requirements
- **C. Disaccharide Composition:** The chromatogram of the enzymatically digested *Sample solution* as obtained in the test for *Limit of Nonspecific Disaccharides* shows three main peaks corresponding to dehydrated glucuronic acid-[1→3]-chondrosamine-4-sulfated (ΔDi-4S), dehydrated glucuronic acid-[1→3]-chondrosamine-6-sulfated (ΔDi-6S), and nonsulfated dehydrated glucuronic acid-[1→3]-chondrosamine (ΔDi-0S) in the enzymatically digested *Standard solution*. By peak-area response, ΔDi-4S is the most abundant, followed by ΔDi-6S, with ΔDi-0S being the least abundant of the three. The ratio of the peak response of the ΔDi-4S to the ΔDi-6S is NLT 1.0.
- **D. Specific Rotation:** Meets the requirements for *Optical Rotation* ⟨781S⟩*, Specific Rotation* in *Specific Tests*

**COMPOSITION**
- **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 100 mg of dried Chondroitin Sulfate Sodium to a 100-mL volumetric flask, dissolve in 30 mL of water, and dilute with water to volume.
**Diluent:** Weigh about 297 mg of monobasic potassium phosphate, 492 mg of dibasic potassium phosphate, and 250 mg of polysorbate 80, and transfer to a 1-L beaker. Dissolve in 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. Degas before use.
**Endpoint detection:** Turbidimetric with a photoelectric probe
**Analysis**
**Samples:** *Standard solutions*, *Sample solution*, and *Diluent*
Transfer 5.0 mL each of the *Standard solutions* 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 phototrode either at 420, 550, or 660 nm. Set the instrument to zero in absorbance mode. Titrate with *Titrant* using the phototrode 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 chondroitin sulfate sodium in the portion of Chondroitin Sulfate Sodium taken:

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

$C$ = concentration of chondroitin sulfate sodium in the aliquot of the *Sample solution*, obtained from the regression equation (mg/mL)
$C_U$ = concentration of Chondroitin Sulfate Sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–105.0% on the dried basis

**IMPURITIES**
- **Residue on Ignition** ⟨**281**⟩**:** 20.0%–30.0%
- **Chloride and Sulfate** ⟨**221**⟩**,** *Chloride***:** NMT 0.50%; a 0.10-g portion shows no more chloride than corresponds to 0.7 mL of 0.020 N hydrochloric acid.
- **Chloride and Sulfate** ⟨**221**⟩**,** *Sulfate*
**Sample solution:** Dissolve 200 mg in 40 mL of water. Add 10 mL of a 30-mg/mL solution of cetylpyridinium chloride, pass through a filter, and use a 25-mL portion of the filtrate.
**Acceptance criteria:** NMT 0.24%; the *Sample solution* shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid.
- **Electrophoretic Purity**
[**Caution—**Voltages used in electrophoresis can readily deliver a lethal shock. The hazard is increased by the use of aqueous buffer solutions and the possibility of working in damp environments. The equipment, with the possible exception of the power supply, should be enclosed in either a grounded metal case or a case made of insulating material. The case should have an interlock that deenergizes the power supply when the case is opened, after which reactivation should be prevented until activation of a reset switch is carried out. High-voltage cables from the power supply to the apparatus should preferably be a type in which a braided metal shield completely encloses the insulated central conductor, and the shield should be grounded. The base of the apparatus should be grounded metal or contain a grounded metal rim that is constructed

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