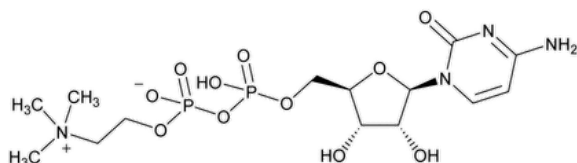


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Add the following:

▲Citicoline



$C_{14}H_{26}N_4O_{11}P_2$ 488.33

5'-O-[Hydroxy({[2-(trimethylammonio)ethoxy]phosphinato}oxy)phosphoryl]cytidine;

Cytidine 5'-diphosphocholine CAS RN®: 987-78-0.

DEFINITION

Citicoline contains NLT 98.0% and NMT 102.0% of citicoline ($C_{14}H_{26}N_4O_{11}P_2$), calculated on the dried basis.

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197K
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: 10 mM [tetrabutylammonium hydroxide](#) and 15 mM [dibasic sodium phosphate anhydrous](#), adjusted with 20% [phosphoric acid](#) to a pH of 6.0

Standard solution: 25 µg/mL of [USP Citicoline RS](#) in [water](#)

Sample solution: 25 µg/mL of Citicoline in [water](#)

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing [L96](#)

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates for citicoline

Tailing factor: NMT 2.0 for citicoline

Relative standard deviation: NMT 2.0% for citicoline

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of citicoline ($C_{14}H_{26}N_4O_{11}P_2$) in the portion of Citicoline taken:

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

r_U = peak response of citicoline from the *Sample solution*

r_s = peak response of citicoline from the *Standard solution*

C_s = concentration of [USP Citicoline RS](#) in the *Standard solution* (mg/mL)

C_u = concentration of Citicoline in the *Sample solution* (mg/mL)

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

IMPURITIES

• [CHLORIDE AND SULFATE \(221\), Chloride](#)

Standard solution: 0.20 mL of 0.010 N [hydrochloric acid](#)

Sample: 0.415 g of Citicoline

Acceptance criteria: NMT 0.017%

• [CHLORIDE AND SULFATE \(221\), Sulfate](#)

Standard solution: 0.20 mL of 0.010 M [sulfuric acid](#)

Sample: 0.96 g of Citicoline

Acceptance criteria: NMT 0.020%

• LIMIT OF PHOSPHATE

Standard phosphoric acid solution: To a 1-L volumetric flask transfer 0.358 g of [potassium dihydrogen phosphate](#), previously dried to constant mass in a desiccator (silica gel) at room temperature. Add 10 mL of diluted [sulfuric acid](#) (3 in 10), and dilute with [water](#) to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with [water](#) to volume. Each milliliter of this solution contains 0.025 mg of phosphoric acid (as PO_4^{3-}).

Ammonium molybdate–sulfuric acid TS: Dissolve 1.0 g of [ammonium molybdate](#) in diluted [sulfuric acid](#) (3 in 20) to make 40 mL. (Prepare at time of use.)

Standard solution: Transfer 4.0 mL of *Standard phosphoric acid solution* to a 20-mL screw-capped tube, and dilute with [water](#) to 10.0 mL.

Sample solution: Transfer 0.1 g of Citicoline to a 20-mL screw-capped tube, and dilute with [water](#) to 10.0 mL.

Analysis: To each *Standard solution* and *Sample solution* add 1.0 mL of *Ammonium molybdate–sulfuric acid TS* and 0.5 mL of [aminonaphtholsulfonic acid TS](#), mix well, and allow to stand at 20° for 30 min. Transfer 2.0 mL of each solution to separate 10-mL volumetric flasks, and dilute with [water](#) to volume. Determine the absorbance of the solutions at 730 nm.

Calculate the percentage of free phosphate in the portion of Citicoline taken:

$$\text{Result} = (A_u/A_s) \times (C_s/C_u) \times 100$$

A_u = absorbance of the *Sample solution*

A_s = absorbance of the *Standard solution*

C_s = concentration of phosphoric acid in the *Standard solution* (mg/mL)

C_u = concentration of Citicoline in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% on the dried basis

• RELATED COMPOUNDS

Mobile phase and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 2.5 µg/mL of [USP Citicoline RS](#) in [water](#)

Quantitative limit solution: 1 µg/mL of [USP Citicoline RS](#) diluted from the *Standard solution*

Sample solution: 2.5 mg/mL of Citicoline in [water](#)

System suitability

Samples: *Standard solution* and *Quantitative limit solution*

Suitability requirements

[NOTE—The run time for the *Sample solution* should be NLT 4 times the retention time of citicoline.]

Column efficiency: NLT 2000 theoretical plates for citicoline, *Standard solution*

Signal-to-noise ratio: NLT 5 for the citicoline peak, *Quantitative limit solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate separately the percentages of cytidine 5'-diphosphoethanolamine, uridine 5'-diphosphocholine, and cytidine 5'-dihydrogenphosphate in the portion of Citicoline taken: