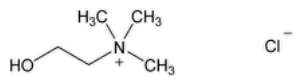


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Choline Chloride



$C_5H_{14}ClNO$ 139.62
(2-Hydroxyethyl)trimethylammonium chloride;
2-Hydroxy-*N,N,N*-trimethylethanaminium chloride CAS RN®: 67-48-1.

Change to read:

DEFINITION

Choline Chloride contains NLT ▲97.0%▲ (USP 1-Aug-2023) and NMT 100.5% of choline chloride ($C_5H_{14}ClNO$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS** (197), *Infrared Spectroscopy*: 197K
- **B. IDENTIFICATION TESTS—GENERAL** (191), *Chemical Identification Tests, Chloride*: A solution (1 in 20) meets the requirements.

Add the following:

▲ **C.** The retention times of the choline and chloride peaks of the *Sample solution* correspond to those of *Standard solution B*, as obtained in the test for *Content of Choline*.▲ (USP 1-Aug-2023)

ASSAY

Delete the following:

▲ **PROCEDURE**▲ (USP 1-Aug-2023)

Add the following:

▲ **CONTENT OF CHOLINE**

[NOTE—To avoid artifact peaks due to salt residues, use polypropylene vessels and vials, and avoid the use of glassware wherever possible.
The pH of the *Buffer solution* can be measured offline to avoid contamination with salt residues.]

Buffer solution: 6.2 g/L of [ammonium acetate](#) in [water](#), adjusted with [glacial acetic acid](#) to a pH of 4.7

Solution A: *Buffer solution* and [acetonitrile](#) (20:80)

Solution B: *Buffer solution*, [water](#), and [acetonitrile](#) (20:30:50)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
20	0	100
22	0	100
26	85	15
28	90	10
35	90	10

Diluent: *Buffer solution* and [acetonitrile](#) (30:70)

System suitability solution: 0.01 mg/mL of [USP Choline Related Compound A RS](#) and 0.1 mg/mL of [USP Choline Chloride RS](#) in *Diluent*

Standard solution A: 0.08 mg/mL of [USP Choline Chloride RS](#) in *Diluent*

Standard solution B: 0.10 mg/mL of [USP Choline Chloride RS](#) in *Diluent*

Standard solution C: 0.12 mg/mL of [USP Choline Chloride RS](#) in *Diluent*

Sample solution: 0.1 mg/mL of Choline Chloride in *Diluent*

Blank: *Diluent*

Chromatographic system

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

Mode: LC

Detector: Charged aerosol detector (CAD). Use settings as follows or optimize according to manufacturer's recommendations to achieve optimal signal-to-noise ratio.

Nebulizer gas: Nitrogen

Evaporator temperature: 35°

Filter constant: 3.6 s

Range: 100 pA

Column: 4.6-mm × 15-cm; 5-μm packing L122

Column temperature: 30°

Flow rate: 0.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution B*

[NOTE—The relative retention times of choline related compound A, choline, and chloride are 0.84, 1.00, and 2.10, respectively.]

Suitability requirements

Resolution: NLT 1.5 between choline related compound A and choline peaks, *System suitability solution*

Relative standard deviation: NMT 5% for choline and chloride peaks, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Plot the choline peak areas against the choline chloride concentrations, in mg/mL, in the three *Standard solutions* and establish a calibration curve by least-squares regression. The correlation coefficient for the regression line is NLT 0.99. Inject the *Sample solution* three times and obtain the average value of choline peak areas. Using this average choline peak area, determine the concentration from the calibration curve (*C*), in mg/mL, of choline chloride in the *Sample solution*.

Calculate the percentage of choline ($C_5H_{14}NO^+$) in the portion of Choline Chloride taken:

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

C = concentration of choline chloride in the *Sample solution* (mg/mL), determined from the calibration curve

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

M_{r1} = molecular weight of choline, 104.17

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

Acceptance criteria: 72.2%–74.7% on the anhydrous basis▲ (USP 1-Aug-2023)

Add the following:

▲• CONTENT OF CHLORIDE

Buffer solution, Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Standard solution A, Standard solution B, Standard solution C, Sample solution, Blank, Chromatographic system, and System suitability: Proceed as directed in the *Content of Choline*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Plot the chloride peak areas against the choline chloride concentrations, in mg/mL, in the three *Standard solutions* and establish a calibration curve by least-squares regression. The correlation coefficient for the regression line is NLT 0.99. Inject the *Sample solution* three times and obtain the average value of chloride peak areas. Using this average chloride peak area, determine the concentration from the calibration curve (*C*), in mg/mL, of choline chloride in the *Sample solution*.

Calculate the percentage of chloride (Cl^-) in the portion of Choline Chloride taken:

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

C = concentration of choline chloride in the *Sample solution* (mg/mL), determined from the calibration curve

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

M_{r1} = molecular weight of chloride, 35.45