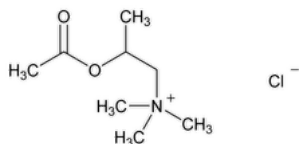


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Methacholine Chloride



$C_8H_{18}ClNO_2$ 195.69

1-Propanaminium, 2-(acetyloxy)-*N,N,N*-trimethyl-, chloride, (±)-;

(±)-(2-Hydroxypropyl)trimethylammonium chloride acetate CAS RN®: 62-51-1; UNII: 0W5ETF9M2K.

DEFINITION

Change to read:

Methacholine Chloride contains NLT 98.0% and NMT 101.0% of methacholine chloride ($C_8H_{18}ClNO_2$), calculated on the ▲anhydrous▲ (USP 1-May-2020) basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** (197), *Infrared Spectroscopy*: 197M or 197A▲ (USP 1-May-2020)

• **B.** [IDENTIFICATION TESTS—GENERAL \(191\)](#), [Chemical Identification Tests, Chloride](#)

Sample solution: 20 mg/mL

Acceptance criteria: Meets the requirements

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

Change to read:

• PROCEDURE

Mobile phase: 0.5 g/L of [methanesulfonic acid](#) in [water](#)

Standard solution: 50 µg/mL of [USP Methacholine Chloride RS](#) in [water](#)

System suitability solution: 10 µg/mL of [USP Acetylcholine Chloride RS](#) in *Standard solution*

Sample solution: 50 µg/mL of Methacholine Chloride in [water](#)

Chromatographic system

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

Mode: IC

Detector: Conductivity

Columns

Guard: 4.0-mm × 50-mm; ▲7-µm▲ (USP 1-May-2020) packing [L77](#)

Analytical: 4.0-mm × 25-cm; ▲7-µm▲ (USP 1-May-2020) packing [L77](#)

▲**Autosampler temperature:** 4°▲ (USP 1-May-2020)

Suppressor: Ion-exchange membrane autosuppressor¹ or a suitable chemical suppression system

Suppressant: Autosuppression

Flow rate: 1 mL/min

Injection volume: 20 µL

▲**Run time:** NLT 2 times the retention time of methacholine▲ (USP 1-May-2020)

System suitability

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

[NOTE—See [Table 1](#) for the relative retention times.]

Suitability requirements

Resolution: NLT ▲1.5▲ (USP 1-May-2020) between acetylcholine and methacholine, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methacholine chloride ($C_8H_{18}ClNO_2$) in the portion of Methacholine Chloride taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of [USP Methacholine Chloride RS](#) in the *Standard solution* (µg/mL)

C_U = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–101.0% on the ▲anhydrous▲ (USP 1-May-2020) basis

IMPURITIES

- [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

Change to read:

- **ORGANIC IMPURITIES**

Mobile phase: 0.5 g/L of [methanesulfonic acid](#) in [water](#)

System suitability solution: 1 mg/mL of [USP Methacholine Chloride RS](#), 1 µg/mL of [USP Acetylcholine Chloride RS](#), ▲and 1 µg/mL of [USP Beta-Methylcholine Chloride RS](#)▲ (USP 1-May-2020) in [water](#)

▲**Sensitivity solution:** 0.5 µg/mL each of [USP Methacholine Chloride RS](#), [USP Acetylcholine Chloride RS](#), and [USP Beta-Methylcholine Chloride RS](#) in [water](#)▲ (USP 1-May-2020)

Standard solution: 1 µg/mL each of [USP Methacholine Chloride RS](#), ▲[USP Acetylcholine Chloride RS](#), and [USP Beta-Methylcholine Chloride RS](#)▲ (USP 1-May-2020) in [water](#)

Sample solution: 1 mg/mL of Methacholine Chloride in [water](#)

Chromatographic system

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

Mode: IC

Detector: Conductivity

Columns

Guard: 4.0-mm × 50-mm; ▲7-µm▲ (USP 1-May-2020) packing [L77](#)▲ (USP 1-May-2020)

Analytical: 4.0-mm × 25-cm; ▲7-µm▲ (USP 1-May-2020) packing [L77](#)

▲**Autosampler temperature:** 4°▲ (USP 1-May-2020)

Suppressor: Ion-exchange membrane autosuppressor¹ or a suitable chemical suppression system

Suppressant: Autosuppression

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: ▲NLT▲ (USP 1-May-2020) 2 times the retention time of methacholine

System suitability

Samples: *System suitability solution* ▲and *Sensitivity solution*▲ (USP 1-May-2020)

[NOTE—See [Table 1](#) for the relative retention times.]

Suitability requirements

Resolution: NLT ▲1.5▲ (USP 1-May-2020) between acetylcholine and methacholine; ▲NLT 2 between acetylcholine and beta-methylcholine, *System suitability solution*

Tailing factor: NMT 1.5 each for acetylcholine, beta-methylcholine, and methacholine, *Sensitivity solution*

Relative standard deviation: NMT 5.0% each for acetylcholine, beta-methylcholine, and methacholine, *Sensitivity solution*▲ (USP 1-May-2020)

Signal-to-noise ratio: NLT ▲10 each for acetylcholine, beta-methylcholine, and methacholine, *Sensitivity solution*▲ (USP 1-May-2020)

Analysis

Samples: *Standard solution* and *Sample solution*

▲Calculate the percentage of beta-methylcholine in the portion of Methacholine Chloride taken:

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

r_U = peak response of beta-methylcholine from the *Sample solution*

r_s = peak response of beta-methylcholine from the *Standard solution*

C_s = concentration of [USP Beta-Methylcholine Chloride RS](#) in the *Standard solution* (µg/mL)

C_u = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)▲ (USP 1-May-2020)

Calculate the percentage of ▲▲ (USP 1-May-2020) acetylcholine in the portion of Methacholine Chloride taken:

Result = $(r_u/r_s) \times (C_s/C_u) \times 100$

r_u = peak response of ▲▲ (USP 1-May-2020) acetylcholine from the *Sample solution*

r_s = peak response of methacholine chloride from the *Standard solution*

C_s = concentration of [USP Methacholine Chloride RS](#) in the *Standard solution* (µg/mL)

C_u = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: See [Table 1](#). ▲The reporting threshold is 0.05%.▲ (USP 1-May-2020)

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Beta-methylcholine	0.6	0.10
Acetylcholine	0.8	0.10
Methacholine	1.0	—

SPECIFIC TESTS

Delete the following:

- ▲• [LOSS ON DRYING \(731\)](#).
Analysis: Dry a sample at 105° for 4 h.
Acceptance criteria: NMT 1.5%▲ (USP 1-May-2020)

Add the following:

- ▲• [WATER DETERMINATION \(921\)](#), [Method I](#), [Method Ia](#): NMT 0.5%▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

- [USP REFERENCE STANDARDS \(11\)](#).
[USP Acetylcholine Chloride RS](#)
▲ [USP Beta-Methylcholine Chloride RS](#)
2-Hydroxy-*N,N,N*-trimethylpropan-1-aminium chloride.
 $C_6H_{16}ClNO$ 153.65▲ (USP 1-May-2020)
[USP Methacholine Chloride RS](#)

¹ Available as Cation Self-Regenerating Suppressor (CSRS) from Dionex Inc., or equivalent.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
METHACHOLINE CHLORIDE	Documentary Standards Support	SM42020 Small Molecules 4
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM42020 Small Molecules 4

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