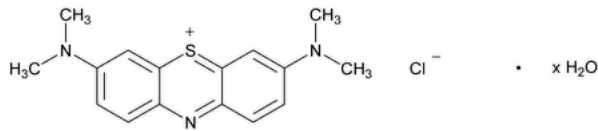


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Methylene Blue

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$C_{16}H_{18}ClN_3S \cdot xH_2O$
Phenothiazin-5-ium, 3,7-bis(dimethylamino)-, chloride, hydrate (1:1:x);
3,7-Bis(dimethylamino)phenothiazin-5-ium chloride hydrate; CAS RN®: 122965-43-9.
Pentahydrate 409.93 CAS RN®: 32680-41-4.
Trihydrate 373.90 CAS RN®: 7220-79-3; UNII: T42P99266K.
Monohydrate 337.90 CAS RN®: 67183-68-0.
Anhydrous 319.85 CAS RN®: 61-73-4; UNII: 8NAP7826UB.

DEFINITION
Methylene Blue contains NLT 97.0% and NMT 103.0% of methylene blue ($C_{16}H_{18}ClN_3S$), calculated on the dried basis.

IDENTIFICATION

- A. [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy](#):** 197A or 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.
- C. [IDENTIFICATION TESTS—GENERAL \(191\)](#), [Chemical Identification Tests, Chloride](#)**
Sample solution: Ignite 50 mg of Methylene Blue with 0.5 g of [anhydrous sodium carbonate](#). Cool and dissolve the residue in 10 mL of 2 N [nitric acid](#). Filter and use 2 mL of the filtrate for performing the test.
Acceptance criteria: Meets the requirements

ASSAY

- PROCEDURE**
All solutions containing methylene blue should be prepared fresh before analysis.
Solution A: 0.1% [trifluoroacetic acid](#) in [water](#)
Solution B: [Acetonitrile](#)
Diluent: *Solution A* and *Solution B* (70:30)
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
25	30	70
32	30	70
33	80	20
38	80	20

Standard solution: 1 mg/mL of [USP Methylene Blue RS](#) in *Diluent*. Stirring and sonication may be necessary for complete dissolution.
Sample solution: 1 mg/mL of Methylene Blue in *Diluent*. Stirring and sonication may be necessary for complete dissolution.

Chromatographic system(See [Chromatography \(621\)](#), [System Suitability](#).)**Mode:** LC**Detector:** UV 246 nm**Column:** 4.6-mm × 10-cm; 3.5-μm packing [L11](#)**Column temperature:** 30°**Flow rate:** 1 mL/min**Injection volume:** 5 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 3.0**Relative standard deviation:** NMT 1.10%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of methylene blue (C₁₆H₁₈ClN₃S) in the portion of Methylene Blue taken:

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

 r_U = peak response of methylene blue from the *Sample solution* r_S = peak response of methylene blue from the *Standard solution* C_S = concentration of [USP Methylene Blue RS](#) in the *Standard solution* (mg/mL) C_U = concentration of Methylene Blue in the *Sample solution* (mg/mL)**Acceptance criteria:** 97.0%–103.0% on the dried basis**IMPURITIES**• [RESIDUE ON IGNITION \(281\)](#): NMT 0.15%• **ORGANIC IMPURITIES****Diluent, Mobile phase, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.**System suitability solution:** 1 mg/mL of [USP Methylene Blue RS](#) and 0.025 mg/mL of [USP Azure B RS](#) in *Diluent***Sensitivity solution:** 0.5 μg/mL of [USP Methylene Blue RS](#) in *Diluent* from the *Standard solution***System suitability****Samples:** *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for azure B and methylene blue are 0.8 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 3.5 between methylene blue and azure B peaks, *System suitability solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Sample:** *Sample solution*

Calculate the percentage of azure B or any unspecified impurity in the portion of Methylene Blue taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response of azure B or any unspecified impurity r_T = sum of all the peak responses**Acceptance criteria:** See [Table 2](#). The reporting threshold is 0.05%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure B ^a	0.8	2.5
Methylene blue	1.0	—
Any unspecified impurity	—	0.10

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities ^b	—	0.5

^a 3-(Dimethylamino)-7-(methylamino)phenothiazin-5-ium chloride.

^b Total impurities does not include azure B.

SPECIFIC TESTS

Change to read:

- [LOSS ON DRYING \(731\)](#).

Analysis: Dry at 105° for 5 h.

Acceptance criteria: 8.0%–▲24.0%▲ (RB 1-Sep-2023)

- [BACTERIAL ENDOTOXINS TEST \(85\)](#): The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Methylene Blue is used can be met. Where the label states Methylene Blue must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Methylene Blue is used can be met.

- [MICROBIAL ENUMERATION TESTS \(61\)](#) and [TESTS FOR SPECIFIED MICROORGANISMS \(62\)](#): The total aerobic microbial count is NMT 10² cfu/g, and the total combined yeasts and molds is NMT 10¹ cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers and protect from light. Store below 30°.
- **LABELING:** Where Methylene Blue must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.
- [USP REFERENCE STANDARDS \(11\)](#).

[USP Azure B RS](#)

3-(Dimethylamino)-7-(methylamino)phenothiazin-5-ium chloride.

C₁₅H₁₆ClN₃S 305.82

[USP Methylene Blue RS](#)

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

Topic/Question	Contact	Expert Committee
METHYLENE BLUE	Documentary Standards Support	SM22020 Small Molecules 2
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM22020 Small Molecules 2

Chromatographic Database Information: [Chromatographic Database](#)

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