Methylene Blue

Type of Posting	Notice of Intent to Revise
Posting Date	30-Jun-2023
Targeted Official Date	To Be Determined, Revision Bulletin
Expert Committee	Small Molecules 2

In accordance with the Rules and Procedures of the Council of Experts and the Pending Monograph Guideline, this is to provide notice that the Small Molecules 2 Expert Committee intends to revise the Methylene Blue monograph.

Based on the supporting data received from a manufacturer awaiting FDA approval, the Expert Committee proposes to revise the Methylene Blue monograph to widen the *Acceptance criteria* in the test for *Loss on Drying* from 8.0%–22.0% to 8.0%–24.0%.

The proposed revision is contingent on FDA approval of a product that meets the proposed monograph specifications. The proposed revision will be published as a Revision Bulletin and an official date will be assigned to coincide as closely as possible with the FDA approval of the associated product.

See below for additional information about the proposed text.[1]

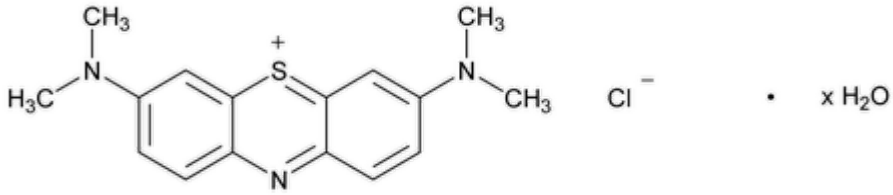
Should you have any questions, please contact Yanyin Yang, Senior Scientist II (301-692-3623 or yanyin.yang@usp.org).

[1] This text is not the official version of a *USP–NF* monograph and may not reflect the full and accurate contents of the currently official monograph. Please refer to the current edition of the *USP–NF* for official text.

USP provides this text to indicate changes that we anticipate will be made official once the product subject to this proposed revision under the Pending Monograph Program receives FDA approval. Once FDA approval is granted for the associated revision request, a Revision Bulletin will be posted that will include the changes indicated herein, as well as any changes indicated in the product's final approval, combined with the text of the monograph as effective on the date of approval. Any revisions made to a monograph under the Pending Monograph Program that are posted without prior publication for comment in the *Pharmacopeial Forum* must also meet the requirements outlined in the USP Guideline on Use of Accelerated Processes for Revisions to the *USP–NF*.

Notice of Intent to Revise
Official: To Be Determined

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 ($C_{16}H_{18}ClN_3S$) 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%

Delete the following:

▲● **Arsenic**▲ (USP 1-Aug-2023)

Delete the following:

▲● **Copper or Zinc**▲ (USP 1-Aug-2023)

● **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
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%▲ (TBD)

- **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 10^2 cfu/g, and the total combined yeasts and molds is NMT 10^1 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.

  $C_{15}H_{16}ClN_3S$ 305.82

  USP Methylene Blue RS

Page Information:

Not Applicable

Current DocID: