Methylene Blue Injection

Type of Posting	Revision Bulletin
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Expert Committee	Chemical Medicines Monographs 2
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the *Chemical Medicines Monographs 2 Expert Committee* has revised the Methylene Blue Injection monograph to accommodate the drug product recently approved by the FDA. The changes listed below were previously published in *Pharmacopoeial Forum 42(1) [Jan. –Mar. 2016].*

- The UV spectrophotometric-based *Assay* procedure is replaced with a stability-indicating liquid chromatography-based procedure. The liquid chromatographic procedure is validated using the X-Bridge Phenyl brand of L11 column. The typical retention time for methylene blue is about 6 min.
- The acceptance criteria under the *Definition* are revised from NLT 9.5 mg/mL and NMT 10.5 mg/mL to NLT 95.0% and NMT 105.0%.
- The visible spectrophotometric-based *Identification A* test is replaced with the UV-Vis absorption spectra match using the revised HPLC *Assay* procedure.
- The TLC-based *Identification B* test is replaced with the retention time agreement as obtained in the revised HPLC *Assay* procedure.
- An *Organic Impurities* test is added. The liquid chromatographic procedure is validated using the X-Bridge Phenyl brand of L11 column. The typical retention time for methylene blue under the conditions specified is about 11 min.
- A *Limit of Azure B* test is added. The liquid chromatographic procedure is validated using the X-Bridge Phenyl brand of L11 column. The typical retention time for methylene blue is about 6 min.
- Storage conditions are added in accordance with the drug product package Insert.
- USP Azure B RS is added under the *USP Reference Standards <11>* section.

Minor editorial changes have been made to update the monograph to the current *USP* style.

The Methylene Blue Injection Revision Bulletin supersedes the currently official monograph and will become official on November 1, 2016. The Revision Bulletin will be incorporated in the *First Supplement* to *USP 40–NF 35.*

Should you have any questions, please contact Sujatha Ramakrishna, Ph.D., MBA. Senior Scientific Liaison (301–816–8349 or sxr@usp.org).

Methylene Blue Injection

DEFINITION

Change to read:

Methylene Blue Injection is a sterile solution of Methylene Blue in Water for Injection. It contains •NLT 95.0% and NMT 105.0%• (RB 1-Nov-2016) of the labeled amount of methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$).

IDENTIFICATION

Delete the following:

•• **A.** The visible absorption spectrum of the solution used for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of the *Standard solution* used in the *Assay*, concomitantly measured.• (RB 1-Nov-2016)

Add the following:

•• **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.• (RB 1-Nov-2016)

Delete the following:

•• **B. THIN-LAYER CHROMATOGRAPHIC TEST**
Adsorbent: 0.25-mm layer of chromatographic silica gel
Standard solution: 5 mg/mL of USP Methylene Blue RS in a mixture of methanol and water (1:1)
Sample solution: Dilute a portion of the Injection with an equal volume of methanol
Application volume: 1 µL
Developing solvent system: Alcohol, acetic acid, and water (3:3:4)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram, using the *Developing solvent system*, until the solvent front has moved about 10 cm above the line of application. Remove the plate from the developing chamber and allow the solvent to evaporate.
Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.• (RB 1-Nov-2016)

Add the following:

•• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.• (RB 1-Nov-2016)

ASSAY

Delete the following:

•• **PROCEDURE**
Standard solution: 2 µg/mL of USP Methylene Blue RS in diluted alcohol
Sample solution: 2.4 µg/mL of methylene blue trihydrate (2 µg/mL of anhydrous methylene blue) in diluted alcohol
Instrumental conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: UV-Vis
Analytical wavelength: Maxima at about 663 nm
Cell: 1 cm
Blank: Diluted alcohol
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the quantity, in mg, of methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) in each mL of the Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times L$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Methylene Blue RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methylene blue in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of methylene blue trihydrate, 373.90
M_{r2} = molecular weight of anhydrous methylene blue, 319.85
L = label claim (mg/mL)

Acceptance criteria: 9.5–10.5 mg• (RB 1-Nov-2016)

Add the following:

•• **PROCEDURE**
All solutions containing methylene blue are recommended to be freshly prepared prior to analysis.
Solution A: 0.1% trifluoroacetic acid in water
Solution B: Acetonitrile
Diluent: *Solution A* and *Solution B* (70:30)
Mobile phase: *Solution A* and *Solution B* (75:25)
Standard solution: 100 µg/mL of USP Methylene Blue RS in *Diluent*
Sample solution: Nominally 100 µg/mL of methylene blue in *Diluent* from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 10-cm; 3.5-µm packing L11
Temperatures
Autosampler: 5°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) in the portion of Injection taken:

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

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* (µg/mL)
C_U = nominal concentration of methylene blue in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of methylene blue trihydrate, 373.90
M_{r2} = molecular weight of methylene blue anhydrous, 319.85

Acceptance criteria: 95.0%–105.0%● (RB 1-Nov-2016)

IMPURITIES

Add the following:

•● **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: For analyzing azure A, azure C, and unspecified impurities, see *Table 1*.

Table 1

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

System suitability solution: 1 mg/mL of USP Methylene Blue RS and 0.03 mg/mL of USP Azure B RS in *Diluent*

Standard solution: 1 µg/mL of USP Methylene Blue RS in *Diluent*

Sensitivity solution: 0.25 µg/mL of USP Methylene Blue RS in *Diluent* from the *Standard solution*

Sample solution: Nominally equivalent to 500 µg/mL of methylene blue (anhydrous) in *Diluent* from Injection

System suitability

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

Suitability requirements

Resolution: NLT 3.5 between the methylene blue and azure B peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Injection taken:

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

r_U = peak response of each impurity 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* (µg/mL)
C_U = nominal concentration of methylene blue in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Injection taken:

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

r_U = peak response of any unspecified impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure C	0.32	0.20
Azure A	0.52	0.20
Azure B[a]	0.82	3.0
Methylene blue	1.00	—
Any unspecified impurity	—	0.20
Total impurities	—	5.0

[a] Azure B is quantitated using the test for *Limit of Azure B* and is included in the table for identification purposes.

● (RB 1-Nov-2016)

Add the following:

•● **LIMIT OF AZURE B**

Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 3 µg/mL of USP Methylene Blue RS in *Diluent*

System suitability: Proceed as directed in the *Assay* using the *Standard solution* from the *Assay*.

[NOTE—The relative retention times for azure B and methylene blue are 0.70 and 1.00, respectively.]

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of azure B in the portion of Injection taken:

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

r_U = peak response of azure B 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*
C_U = nominal concentration of methylene blue in the *Sample solution*

Acceptance criteria: NMT 3.0%● (RB 1-Nov-2016)

SPECIFIC TESTS

• **pH** ⟨**791**⟩**:** 3.0–4.5

Change to read:

• •**BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:**● (CN 1-MAY-2016) NMT 2.5 USP Endotoxin Units/mL

Change to read:

• **Other Requirements:** It meets the requirements in • *Injections and Implanted Drug Products* ⟨1⟩.● (CN 1-May-2016)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. •Store at room tempera-

ture, protected from light. Do not refrigerate or freeze.● (RB 1-Nov-2016)

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩
  ●USP Azure B RS
  3-(Dimethylamino)-7-(methylamino)phenothiazin-5-ium chloride.

$C_{15}H_{16}ClN_3S$ 305.82● (RB 1-Nov-2016)
USP Endotoxin RS
USP Methylene Blue RS