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Methylene Blue Injection

Change to read:

DEFINITION

Methylene Blue Injection is a sterile solution of Methylene Blue in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of methylene blue ▲trihydrate▲ (USP 1-Aug-2022) ($C_{16}H_{18}ClN_3S \cdot 3H_2O$).

IDENTIFICATION

• **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*.

• **B.** 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**

All solutions containing methylene blue ▲should▲ (USP 1-Aug-2022) 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 ▲trihydrate▲ (USP 1-Aug-2022) ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) 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%

IMPURITIES

Change to read:

• **Organic Impurities**

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

Mobile phase: ▲▲ (USP 1-Aug-2022) 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 ▲▲ (USP 1-Aug-2022) 500 µg/mL of methylene blue ▲▲ (USP 1-Aug-2022) 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 ▲degradation product▲ (USP 1-Aug-2022) 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 ▲specified degradation product▲ (USP 1-Aug-2022) 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 ▲degradation product▲ (USP 1-Aug-2022) in the portion of Injection taken:

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

r_U = peak response of any unspecified ▲degradation product▲ (USP 1-Aug-2022) from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. ▲The reporting threshold is▲ (USP 1-Aug-2022) 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure C▲[a]▲ (USP 1-Aug-2022)	0.32	0.20
Azure A▲[b]▲ (USP 1-Aug-2022)	0.52	0.20
Azure B[c]	0.82	3.0
Methylene blue	1.00	—
Any unspecified ▲degradation product▲ (USP 1-Aug-2022)	—	0.20
Total ▲degradation products[d]▲ (USP 1-Aug-2022)	—	5.0

[a] 3-Amino-7-(methylamino)phenothiazin-5-ium chloride.

[b] 3-Amino-7-(dimethylamino)phenothiazin-5-ium chloride.

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

[d] Total degradation products include the sum of the results from the tests for *Organic Impurities* and *Limit of Azure B*.

Change to read:

• **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: *Standard solution* and *Sample 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* ▲(µg/mL)▲ (USP 1-Aug-2022)

C_U = nominal concentration of methylene blue in the *Sample solution* ▲(µg/mL)▲ (USP 1-Aug-2022)

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.0–4.5

Change to read:

• **Bacterial Endotoxins Test** ⟨85⟩**:** ▲Meets the requirements▲ (USP 1-Aug-2022)

Add the following:

▲• **Sterility Tests** ⟨71⟩**:** Meets the requirements▲ (USP 1-Aug-2022)

• **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass. Store at ▲controlled▲ (USP 1-Aug-2022) room temperature, protected from light. Do not refrigerate or freeze.

Add the following:

▲• **Labeling:** The label to indicate for "Intravenous use only".▲ (USP 1-Aug-2022)

• **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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
METHYLENE BLUE INJECTION	Documentary Standards Support	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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