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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