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Methylene Blue Compounded Injection, Veterinary

DEFINITION

Methylene Blue Compounded Injection, Veterinary, 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 ($C_{16}H_{18}ClN_3S$). Prepare Methylene Blue Compounded Injection, Veterinary, 10 mg/mL, as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Methylene Blue	5 g
Sterile Water for Injection or Sodium Chloride Injection (0.9%), a sufficient quantity to make	500 mL

Dissolve an accurately weighed quantity of *Methylene Blue* in *Sterile Water for Injection* or *Sodium Chloride Injection (0.9%)*, and dilute to volume with mixing. Sterilize by a suitable means, such as sterile filtration or autoclaving.

IDENTIFICATION

• **A.** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.

• **B.** **Thin-Layer Chromatographic Identification Test** ⟨201⟩

Diluent: Methanol and water (1:1)

Standard solution: 5 mg/mL of USP Methylene Blue RS in *Diluent*

Sample solution: Dilute a portion of 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 until the solvent front has moved about 10 cm above the line of application. Remove the plate from the 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*.

ASSAY

• **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 ($C_{16}H_{18}ClN_3S$) 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, 319.86

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **pH ⟨791⟩:** 3.0–4.5
- **Bacterial Endotoxins Test ⟨85⟩:** NMT 0.17 USP Endotoxin Units/mg of methylene blue
- **Sterility Tests ⟨71⟩:** Meets the requirements
- **Other Requirements:** It meets the requirements in *Labeling ⟨7⟩, Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Package in single-dose containers, preferably of Type I glass. Store at controlled room temperature, protected from light.

Change to read:

- **Beyond-Use Date:** ▲In the absence of performing and completing a sterility and endotoxin test, the storage conditions in *Pharmaceutical Compounding – Sterile Preparations ⟨797⟩, 14.3 Establishing a BUD for a CSP* apply.▲ (CN 1-Nov-2023)
- **Labeling:** Label it to indicate that it is to be discarded after its *Beyond-Use Date* and to indicate the nominal content of methylene blue in the Injection and whether it was prepared in Sterile Water for Injection or in Sodium Chloride Injection (0.9%). Label it to indicate that it is for veterinary use only and to state the *Beyond-Use Date*.
- **USP Reference Standards ⟨11⟩**
  USP Methylene Blue RS

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

Topic/Question	Contact	Expert Committee
METHYLENE BLUE COMPOUNDED INJECTION, VETERINARY	Brian Serumaga Science Program Manager	CMP2020 Compounding 2020

Chromatographic Database Information: Chromatographic Database

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