Letco Medical, LLC.

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# CERTIFICATE OF ANALYSIS

| | | | | | | |
|---|---|---|---|---|---|---|
| **Letco Item:** | 697831 | 697832 | | | | |
| **Letco Lot:** | 2410220006 | 2410220007 | | | | |

| DESCRIPTION | SOURCE LOT | DATE OF MANUFACTURE | EXPIRATION DATE |
|---|---|---|---|
| Methylene Blue USP | 0024 | May 2024 | May 2029 |

| | TEST | SPECIFICATION | RESULT | METHOD |
|---|---|---|---|---|
| | Description | Dark green or blue crystals or crystalline powder having bronze-like luster. | Complies | USP |
| | **Identification by** | | **Complies** | |
| * | IR | The infrared absorption spectrum of the sample must be concordant with the standard. | Complies | USP |
| | HPLC | The RT of the major peak of the sample solution corresponds to that of the standard solution as obtained in the assay | Complies | USP |
| | Chloride | Meets the requirements. | Complies | USP |
| | | | | |
| | Loss on drying (at 105ºC for 5 hrs) | 15.0 – 22.0% w/w | 19.4% | USP |
| | Residue on Ignition | Not more than 0.15% w/w | 0.07% | USP |
| | **Related substances by HPLC** | | **Complies** | |
| | Azure-B | Not more than 2.5% | 1.4% | USP |
| | Any Unspecified Impurity | Not more than 0.10% | 0.07% | USP |
| | Total Impurities (except Azure-B) | Not more than 0.5% | 0.07% | USP |
| | | | | |
| | Assay by HPLC (On dried basis) | Not less than 97.0% w/w and Not more than 103.0% w/w | 99.8% | USP |
| | **N-Methyl aniline and N,N-Dimethyl aniline contents by HPLC** | | **Complies** | |
| | N-Methyl aniline | Not more than 0.10% w/w | < 0.0004% | Mfr. In-house |
| | N,N-Dimethylaniline | Not more than 0.10% w/w | < 0.0004% | Mfr. In-house |
| | **# Genotoxic impurities by LC-MS/MS (ppm)** | | **Complies** | |
| | Methylene blue stage-1 | Not more than 0.5 ppm | < 0.0165 ppm | Mfr. In-house |
| | N,N-Dimethyl-4-Nitrosoaniline | Not more than 0.5 ppm | < 0.0165 ppm | Mfr. In-house |
| | DMPDA.2HCl | Not more than 0.5 ppm | < 0.0165 ppm | Mfr. In-house |
| | **Residual Solvents by GC-HS** | | **Complies** | |
| | Dichloromethane | Not more than 600 ppm | < 9 ppm | Mfr. In-house |
| | Isopropyl alcohol | Not more than 5000 ppm | < 62 ppm | Mfr. In-house |

**Letco Medical certifies that this is a true duplication of the manufacturer's original certificate of analysis**

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| | | | | |
|---|---|---|---|---|
| | **♦ Microbial Enumeration tests** | | **Complies** | |
| | ♦ Total aerobic microbial count (TAMC) | Not more than 100 cfu/g | < 10 cfu/g | USP |
| | ♦ Total yeast and mold count (TYMC) | Not more than 10 cfu/g | < 10 cfu/g | USP |
| | **Tests for Specified Microorganisms** | | **Complies** | |
| | Escherichia coli | Should be absent | Absent | --- |
| | Salmonella | Should be absent | Absent | --- |
| | Pseudomonas aeruginosa | Should be absent | Absent | --- |
| | Staphylococcus aureus | Should be absent | Absent | --- |
| | Clostridia | Should be absent | Absent | --- |
| | Candida albicans | Should be absent | Absent | --- |
| | Bile tolerant gram negative bacteria | Should be absent | Absent | --- |
| * | ♦ **$** Bacterial endotoxins test | Not more than 2.5 EU/mL | < 2.5 EU/mL | USP |
| | **♦ Elemental Impurities by ICP-MS** | | **Complies** | |
| | Copper | Not more than 200 ppm | < 15.152 ppm | Mfr. In-house |
| | Zinc | Not more than 100 ppm | < 7.576 ppm | Mfr. In-house |
| | Manganese | Not more than 10 ppm | < 0.758 ppm | Mfr. In-house |

## ADDITIONAL INFORMATION

** Verification testing performed by Letco Medical is represented by an asterisk.*

This product is Pentahydrate form.
CAS No.: 32680-41-4

Per the manufacturer:
♦ Marked tests are performed at external laboratories.
# In line with ICH M7, Genotoxic impurities by LC-MS/MS is tested skip wise on every 10th batch.
**$** Limit is adopted from Methylene Blue USP monograph USP41 NF36.

Chemical Names:
Methylene Blue: Phenothiazin-5-ium, 3,7-bis (dimethylamino) chloride
Azure-B: 3-(Dimethyl amino)-7-(methyl amino) phenothiazin-5-ium chloride
DMPDA.2HCl: N,N-Dimethyl-1,4-phenylenediamine dihydrochloride
Stage-1: 2-Amino-5-(N,N-dimethylamino) phenyl-1-thiosulfonic acid

Per the manufacturer:
This batch / lot complies with corresponding Manufacturer specifications and USP current edition.
This batch / lot was manufactured in compliance with cGMP.

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This product was manufactured by Pharmazell (India) Private Limited, Plot No. 115, Visakha Pharmacity Limited, SEZ, Parawada, Anakapalli, Andhra Pradesh 531019, India (IND)

**LETCO QA REVIEW:** Robin Owens

Digitally signed by Robin Owens
DN: cn=Robin Owens, o=Fagron, ou=Quality Assurance, email=Robin.Owens@Fagron.US, c=US
Date: 2025.03.13 15:21:52 -05'00'

**Letco Medical certifies that this is a true duplication of the manufacturer's original certificate of analysis**