1. **DESCRIPTION**

*Specification:* White crystalline powder.

1. **IDENTIFICATION**
	1. **Phosphocreatine (IR spectrum)**

*Specification:* Conforms to the standard.

The IR spectrum of potassium bromide demonstrates maximum dispersion at the same wavelengths as the equally prepared reference sample.

* 1. **Creatine**

*Specification:* Red coloring should appear.

**Procedure.** 2 ml of an aqueous solution containing 10 mg of creatine phosphate is heated in a boiling water bath for 10 minutes, 2 ml of Alpha-Naphthol and diacetyl solution are added.

**α-Naphthol Solution.** Dissolve 0.10 g of 1-naphthol in 3 ml of a 15% w/v solution of sodium hydroxide and dilute to 100 ml with water. Prepare immediately before use.

* 1. **Phosphorus**

*Specification:* Blue coloring should appear.

**Procedure.** 10 mg of creatine phosphate is dissolved in 25 ml of 0.5 M sulfuric acid, heated in a boiling water bath for 10 minutes, cooled and added ml of reagent A and ml of reagent B, stirred and left for a while. Blue coloring should appear.

**Reagent A:** 5 g of ammonium molybdate is placed in a 100 ml volumetric flask, dissolved in a 1 M solution of sulfuric acid and the volume in the flask is brought to the mark with the same solvent.

**Reagent B:** 200 mg of p-methylaminophenol sulfate is dissolved in 100 ml of water and 20 g of sodium sulfate is added. The reagent should be stored in tightly closed containers and used for 1 month.

* 1. **Sodium**

*Specification:* Yellow coloring.

**Procedure.** When the drug is introduced into the flame of the burner, a yellow staining appears, characteristic of sodium.

1. **HOMOGENEITY BY MASS OF VIAL CONTENTS**

*Specification:* Unit values should be within ±10 % of the average mass.

**Procedure.** The determination is carried out on 20 vials, the stopper is removed on each vial and weighed with an accuracy of 0.01 g. the contents of the vial are removed, the vial and stopper are washed with purified water and dried in a drying cabinet at 105 °C. The bottle with the stopper is weighed again and the weight of the contents of each bottle is calculated based on the difference between the weight of the bottle with the drug and the empty bottle.

1. **TRANSPARENCY**

*Specification:* The solution must be transparent.

**Procedure.** Prepare a solution of the drug by dissolving the contents of one vial in 50 ml of water and compare it with water.

1. **pH**

*Specification:* 8.0 - 9.0.

**Procedure.** Prepare a solution of the drug by dissolving the contents of one vial 50 ml of water. Take 5 ml of this solution and determine the pH value using a pH-meter.

1. **PARTICULATE CONTAMINATION**
	1. **Visible particles**

*Specification:* There must be no visible particles.

* 1. **Invisible particles**

*Specification:* NMT 10 µm 6000 per container

 NMT 25 µm 600 per container

**Environment suitability test.** Determine the particulate contamination of 5 samples of particle-free water, each of 5 mL, according to the method described below. If the number of particles of 10 µm or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

**Preparation of Particle-free Water:** Filter water R through a membrane with a pore size of 0.22 µm.

**Procedure.** Prepare a solution of the drug by dissolving the contents of one vial 50 ml of water. Eliminate gas bubbles by appropriate measures such as allowing the sample to stand for 2 min or sonication.

Remove 4 portions, each of not less than 5 ml., and count the number of particles equal to or greater than 10 µm and 25 µm. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

1. **SOLUBILITY**

*Specification:* The contents of one bottle should completely dissolve in a bottle with a solvent to form a transparent solution practically free of foreign particles.

**Procedure.** Dissolve 1 vial of drug in 50 ml water for injection.

1. **IMPURITIES**

*Specification:* Sum of impurities: NMT 5 %

Expressed as the sum of creatine, creatinine and creatinine phosphate.

**Chromatograph conditions.**

**Column:** Lichrosorb RP 18 (10 microns),4.0x250mm (or similar);

Flow rate: 1.5 ml/min;

**Wavelength:** 220 nm;

**Injected volume:** 10 µl;

**Mobile phase:**  0.005 M tetrabutylammonium phosphate solution, pH – 7.

**0.005 M tetrabutylammonium phosphate solution.** 1.7 g of tetrabutylammonium hydrophosphate is placed in a measuring flask with a capacity of 1000 ml, dissolved in 900 ml of water, adjusted pH - 7 with a solution of sodium phosphate 20% and adjusted the volume in the flask with water to the mark.

**Standard solution A.** About 5 mg (exact weight) of creatine, about 5 mg (exact weight) of creatinine and 13 mg creatinine phosphate are placed in a 100 ml volumetric flask, dissolved in water and the volume in the flask is brought to the mark with the same solvent and mixed.

**Standard solution B.** Pour 2.0 ml of the test solution into a 100 ml volumetric flask, bring to the volume with water.

**Test solution.** About 50 mg (exact weight) of the drug is placed in a measuring flask with a capacity of 10 ml, dissolved in water and the volume in the flask is brought to the mark with the same solvent, mixed.

**Procedure.** The standard solution is chromatographed in five repetitions, determining:

 - The resolution between the peaks of creatine and creatinine on the chromatogram of the standard solution is a fraction to be at least 2.5;

 -% of the relative standard deviation is calculated by the peak areas of each impurity, calculated for 5 injections, should be no more than 15%;

 - The coefficient of the peak asymmetry:

 Peak creatine: no more than 1.4;

 Peak creatinine: no more than 1.9;

 Peak creatinine phosphate: no more than 1.3

After checking the suitability of the chromatographic system, 3 injections of a standard solution A and 2 injections of both the test solution and the standard solution B are performed.

Chromatograms are recorded and impurity peaks are determined by their relative retention time:

 for creatine - about 0.14 minutes; for creatinine - about 0.18 minutes;

 for creatinine phosphate - about 0.57 min; for creatine phosphate - 1 min.

The content of creatine, creatinine, creatine phosphate (X, %) is calculated by the formula:

$$X=\frac{A\_{S}×W\_{std}×T\%}{A\_{std}×W\_{s}×10}$$

$A\_{S}$ - is the peak area of the identified impurities on the chromatogram

of the sample solution;

$A\_{std}$ - is the peak area of the identified impurities on the chromatogram of the standard solution;

$W\_{std}$ - is the impurity weight of the standard (mg);

$T\%$ - %purity of the impurity of the standard;

$W\_{s}$ - is the amount of the suspended preparation, mg.

1. **WATER**

*Specification:* 22.0% - 25.0%

**Procedure.** Take 200 mg (exact weight) of the drug and titrate with methanol.

The Karl Fischer apparatus, which is a closed system consisting of one or two automatic burettes and a tightly fitted titration vessel equipped with the necessary electrodes and a magnetic stirrer. The dryness of the air in the system is maintained with a suitable desiccant, the titration tank can be cleaned with nitrogen or dry air.

1. **ASSAY (HPLC)**

*Specification:*95.0% - 110.0%

**Chromatographic conditions:**

**Chromatograph:** For HPLC with UV detector, automatic sampler, pump and integrator (or similar);

**Column:** Symmetry With 18; (5 microns), 4.6x250 mm (or similar);

**Flow rate:** 1.5 ml/min;

**Wavelength:** 210 nm;

**Injected volume:** 10 µl

**Mobile phase:** 0.1 % phosphoric acid solution

**0.1 % phosphoric acid solution.** 1ml of concentrated phosphoric acid is placed in a measuring flask with a capacity of 1000 ml, dissolved in 900 ml of water, and adjusted the volume in the flask with water to the mark.

**Standard solution.** About 50 mg (exact weight) of creatine phosphate (company standard) is placed in a measuring flask with a capacity of 100 ml, dissolved in water and the volume in the flask is brought to the mark with the same solvent and mixed. Prepare the solution before use.

**Test solution.** About 50 mg (exact weight) of the drug is placed in a measuring flask with a capacity of 100 ml, dissolved in water and the volume in the flask is brought to the mark with the same solvent, mixed, the solution is prepared before use.

10 µl of the standard solution is injected into the chromatograph 3 times and 10 µl of the test solution.

Creatine phosphate content (mg/vial) is calculated by the formula:

$$X=\frac{A\_{s}×W\_{st}×W\_{m}}{A\_{st}×W\_{s}}$$

$A\_{s}=$ the peak area of creatine phosphate on the chromatogram of the test solution;

$A\_{st}$= the peak area of creatine phosphate on the chromatogram of the standard solution;

$W\_{st}=$ weight of a standard sample of creatine phosphate, mg;

$W\_{s}$= weight of the drug, mg;

$W\_{m}$= the average weight of the contents of the vials, mg.

1. **BACTERIAL ENDOTOXIN**

This procedure is performed as per the SOP “SOP/ML/059”.

1. **STERILITY**

This procedure is performed as per the SOP “SOP/ML/055”

1. **PACKAGING**

Conforms to the approved packaging art work.

1. **LABELLING**

Conforms to the approved labelling art work

|  |  |  |  |
| --- | --- | --- | --- |
| **Signature/ Date** | **PREPARED BY** | **CHECKED BY** | **APPROVED BY** |
|  |  |  |  |  |
| **Officer / Executive QC** | **Sr. Executive/AM QC** | **Head QC** | **Head ML** | **Head QA** |