Title: Procedure for Chemical Analysis of Purified water and Water for Injection Samples

SOP No.:		**Revision No.:**	00
Effective Date:		**Supersedes No.**	Nil
Review Date:		**Page No.**	1 of 9

1.0 Objective

To lay down a procedure for Chemical Analysis of Purified water and water for injection.

2.0 Scope

This Standard Operating Procedure is applicable for pharmaceutical formulation plant.

3.0 Responsibility

Executive/Officer - Microbiology : Shall be responsible to follow the procedure for Microbiological Analysis of Purified water and water for injection.

Head - QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

SOP	: Standard Operating Procedure
QC	: Quality Control
WFI	: Water for injection
M	: Molarity
N	: Normality
G	: Gram
w/v	: Weight by Volume
ml	: Milliliter
mg	: Miligram
VS	: Volumetric Solution
%	: Percentage

5.0 Test Procedure

5.1 Equipments:

5.1.1 Micropipettes

5.1.2 Weighing Balance

5.1.3 Hot Plate

5.1.4 Muffle Furnace

5.2 Materials and reagents:

5.2.1 Test tubes

5.2.2 Micropipettes tips

5.2.3 Tissue paper

5.2.4 Milli Q water/ WFI

5.2.5 Volumetric flasks

5.2.6 Pipettes

5.2.7 Burettes

5.2.8 Conical Flasks

5.2.9 Methyl Red solution

5.2.10 Bromothymol blue solution

5.2.11 Alkaline Potassium Mercuri-Iodide solution

5.2.12 Dilute Ammonium Chloride Solution

5.2.13 Ammonia Buffer pH 10.0

5.2.14 Disodium Edetate

5.2.15 Lead Nitrate

5.2.16 Nitric Acid

5.2.17 Silver Nitrate

5.2.18 Potassium Chloride

5.2.19 Diphenylamine Solution

5.2.20 Sulphuric Acid

5.2.21 Hydrochloric Acid

5.2.22 Barium Chloride

5.2.23 Potassium Permanganate

5.3 **Solution preparation:**

5.3.1 **Preparation of solutions.**

5.3.2 Alkaline Potassium Mercuric-Iodide solution

5.3.2.1 Weigh accurately 3.5 g of Potassium Iodide. Add 1.25 g of Mercuric Chloride dissolved in 80 ml of water; add a cold, saturated solution of Mercuric Chloride in water, with constant stirring until a slight red precipitate remains.

5.3.2.2 Dissolve 12 g of Sodium Hydroxide in the above solution, add a little more of the cold saturated solution of mercuric chloride and sufficient water to produce 100 ml. Allow standing and decanting the clear, supernatant liquid.

5.3.3 0.1 M Silver Nitrate :

5.3.3.1 Preparation :

Weigh accurately 17 g of Silver Nitrate in a 1000 ml clean and dry volumetric flask and dissolve in 100 ml of Milli-Q water/Water for injection and dilute to 1000 ml with Milli-Q water/Water for injection.

5.3.3.2 Standardization:

Weigh accurately 100 mg of Sodium Chloride (NaCl) previously dried to 110^0C for 2 hours and dissolve in 5 ml of Milli-Q water/Water for injection.Add 5 ml of Acetic acid, 50 ml of Methanol, and 0.15 ml of Eosin solution. Stir, preferably with magnetic stirrer, titrate with Silver Nitrate solution. Each ml of Silver Nitrate is equivalent to 0.05844 g of NaCl.

5.3.3.3 Calculation Formula:

$$\text{Molarity} = \frac{\text{Weight of NaCl in g X 0.1}}{\text{Volume of Silver Nitrate used in ml X 0.05844}}$$

5.3.4 Ammonia Buffer pH 10.0:

5.3.4.1 Dissolve 5.4 g of Ammonium Chloride in 20 ml of water, add 35 ml of 10 M Ammonia and dilute with water to 100 ml.

5.3.5 0.01 M Disodium Edetate :

5.3.5.1 Weigh accurately 3.72 g of Disodium Edetate in a 1000 ml clean and dry volumetric flask and dissolve in 100 ml of Milli-Q water/Water for injection and dilute to 1000 ml with Milli-Q water/Water for injection.

5.3.5.2 Weigh accurately 0.08 g of granulated Zinc; dissolve by gentle warming in 12 ml of dilute HCl and 0.1 ml of Bromine water.

5.3.5.3 Boil to remove excess Bromine, cool and add sufficient water to produce 200 ml.

5.3.5.4 Pipette 20 ml of resulting solution into a flask and nearly neutralize with 2 M NaOH.

5.3.5.5 Dilute to about 150 ml with water, add sufficient Ammonia buffer ph 10.0 to dissolve the precipitate and add 5 ml in excess.

5.3.5.6 Add 50 mg of Mordant black II mixture and titrate with Disodium Edentate solution until the solution turns green. Each ml of 0.01 M Disodium Edentate is equivalent to 0.000654 g of Zn.

5.3.5.7 Calculation

$$\text{Molarity} = \frac{\text{Weight of Zn in g X 0.01 X 20}}{\text{ml of 0.01 M EDTA X 0.000654 X 200}}$$

5.3.6 Lead Nitrate Standard (0.1 %):

5.3.6.1 Dissolve 0.4 g Lead Nitrate in water containing 2 ml of Nitric Acid and add sufficient water to produce 250 ml.

5.3.7 Lead Nitrate Standard (1 ppm) :

5.3.7.1 Dilute 1 ml of Lead Nitrate Standard (0.1 %) to 100 ml with Milli-Q water/WFI.Further dilute 1 ml of this solution to 10 ml with Milli-Q water/WFI.

5.3.8 2 M Nitric Acid :

5.3.8.1 Pipette 12.6 ml of concentrate Nitric acid in 60 ml Milli-Q water/WFI and make up to 100 ml with Milli-Q water/WFI.

5.3.8.2 Dissolve 4 g of anhydrous sodium carbonate in 50 ml of water and titrate with the nitric acid solution using methyl orange solution as indicator until the solution becomes radish yellow. Boil for 2 minutes, cool and continue the titration until the reddish yellow colour is restored.

5.3.8.3 1 ml of 1 M nitric acid is equivalent to 0.053 g of NA_2CO_3.

5.3.8.4 Calculation

$$\text{Molarity} = \frac{\text{Weight of anhydrous sodium corbonate in g X 1}}{\text{Vol. of Nitric acid used X 0.053}}$$

5.3.9 Potassium Chloride (10% w/v) :

5.3.9.1 Dissovle 10 g of Potassium Chloride in 60 ml with Milli-Q water/WFI and make up to 100 ml with Milli-Q water/WFI.

5.3.10 Diphenylamine Solution (0.1% w/v):

5.3.10.1 Dissolve 0.1 g of Diphenylamine in 60 ml Milli-Q water/WFI and make up to 100 ml with Milli-Q water/WFI.

5.3.11 Sulphuric Acid 1 M :

5.3.11.1 Pipette 60 ml of Sulphuric Acid in 600 ml Milli-Q water/WFI and make up to 1000 with Milli-Q water/WFI.

5.3.11.2 Standardization :

Weigh accurately about 1.5g of anhydrous sodium corbonate previously heated at 270°C for 1 hour. Dissolve it in 100 ml of water and add 0.1 ml of methyl red solution. Add the acid slowly from a burette with constant stirring until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink colour is no longer affected by continued boiling.

1 ml of 0.5M sulphuric acid is equivalent to 0.05299 g of Na_2CO_3.

5.3.12 Hydrochloric Acid 2 M :

5.3.12.1 Pipette ml of Hydrochloric Acid in 600 ml Milli-Q water/WFI and make up to 100 with Milli-Q water/WFI.

5.3.12.2 Standardization :

Weigh accurately about 1.5g of anhydrous sodium corbonate previously heated at 270°C for 1 hour. Dissolve it in 100 ml of water and add 0.1 ml of methyl red solution. Add the acid slowly from a burette with constant stirring until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink colour is no longer affected by continued boiling.

5.3.12.3 1 ml of 1M sulphuric acid is equivalent to 0.05299 g of Na_2CO_3.

5.3.13 Barium Chloride (10 % w/v) :

5.3.13.1 Dissovle 10 g of Barium Chloride in 60 ml with Milli-Q water/WFI and make up to 100 ml with Milli-Q water/WFI.

5.3.14 Potassium Permanganate 0.02 M :

5.3.14.1 Weigh accurately 3.2 g of Potassium Permanganate in a 1000 ml clean and dry volumetric flask and dissolve in 100 ml of Milli-Q water/Water for injection and dilute to 1000 ml with Milli-Q water/Water for injection, heat on a water-bath for 1 hour, allow to stand for 2 days and filter through wool.

5.3.14.2 Standardization :

To 25.0 ml of the solution in glass-stoppered, add 2g of potassium iodide followed by 10 ml of 1M sulphuric acid. Titrate the liberated iodine with 0.1M sodium thiosulphate using 3 ml of starch solution added towards the end of the titration, as indicator. Perform the blank determination and make necessary correction.

1 ml of 0.1M sodium thiosulphate is equivalent to 0.003161 g of $KMnO_4$.

Store protected from light.

5.4 **Testing Procedure**

5.4.1 **Description**

Check by physical examination. Clear, colourless, odourless and tasteless liquid

5.4.2 **pH**

Refer SOP for Operation and Cleaning of pH meter and measure the pH of water by adding 0.3 mL of saturated KCl in 100 mL of water.

5.4.3 **Conductivity**

Refer SOP for Operation and Cleaning of conductivity meter and measure the conductivity of water.

5.4.4 **Total Organic Carbon**

Refer SOP for Cleaning and Operation of TOC Analyzer.

5.4.5 **Chloride**

To 10 mL of sample, add 1 mL of 2M Nitric Acid and 0.2 mL of 0.1 M Silver Nitrate, the appearance of the solution does not change for at least 15 minutes.

5.4.6 **Sulphates**

To 10 mL of sample, add 0.1 mL of 2M Hydrochloric Acid and 0.1 mL of Barium Chloride Solution. The appearance of the solution does not change for at least 1 hour.

5.4.7 **Nitrate**

To 5 mL of sample in a test tube immersed in ice, add 0.4 mL of a 10% w/v solution of Potassium Chloride, 0.1 mL of Diphenylamine Solution add drop wise with shaking, 5 mL of Sulphuric Acid. Transfer the tube to a water bath at 50°C and allow standing for 15 minutes. Any blue colour in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 mL of nitrate free water and 0.5 mL of nitrate standard solution (2 ppm NO_3).

5.4.8 **Ammonium**

To 20 mL of sample add 1 mL of alkaline potassium mercury iodide solution and allow to stand for 5 minutes, when viewed vertically the solution is not more intensely coloured than a solution prepared at the same time by adding 1 mL of alkaline potassium mercury-iodide solution to a solution containing 2.5 mL of

Ammonium Standard Solution (1 ppm NH_4) and 7.5 mL of Ammonia free water (0.2 ppm).

5.4.9 **Calcium and Magnesium**

To 100 mL of sample, add 2 mL of Ammonia buffer pH 10.0, 50 mg of mordant black II mixture and 0.5 mL of 0.01 M Disodium Edetate, a pure blue colour is produced.

5.4.10 **Heavy Metal**

Determined by Method D on 12 ml of a solution prepared in the following manner.

In a glass evaporating dish evaporate 150 mL to 15 mL on a water bath.

Use Lead Standard Solution (1 ppm) to prepare the standard.

5.8.1.1 Method D

Take two cylinders, one with 12 mL of evaporated water and second with 10 mL of standard solution & 2 mL of the evaporated water and mix. To each of the cylinders add 2 mL of Acetate buffer pH 3.5, mix, add, 1.2 mL of Thioacetamide reagent, allow to stand for 2 minutes. Then view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

12 ml of solution complies with the limit test for heavy metals (0.1 ppm).

5.4.11 **Acidity or alkalinity**

To 10 mL of sample, freshly boiled and cooled in a borosilicate glass flask, add 0.05 mL of methyl red solution, the resulting solution is not red. To 10 mL, add 0.1 mL of Bromothymol Blue solution, the resulting solution is not blue.

5.4.12 **Oxidisable substances**

To 100 ml add 10 ml of 1M sulphuric acid and 0.1 ml of 0.02M potassium permanganate and boil for 5 minutes, the remains faintly pink.

5.4.13 **Residue on evaporation**

5.4.13.1 Calculations

Calculate the **residue** on evaporation as per follows:

Residue on evaporation = (W2-W1) mg

Where,

W1 = weight of empty beaker

W2 = weight of beaker with sample after evaporation

6.0 Forms and Records

6.1 Nil

7.0 Distribution

7.1	Master Copy	:	Documentation Cell (Quality Assurance)
7.2	Controlled Copies	:	Quality Control, Quality Assurance

8.0 History

Date	Revision Number	Reason for Revision