DEPARTMENT OF DRUG ADMINISTRATION

National Medicines Laboratory

ANALYTICAL METHOD VALIDATION COMMITTEE

Sevelamer Carbonate Tablets

Analytical Profile No.: SEVL 075/076/AP047

Sevelamer Carbonate Tablets contain not less than 90 % and not more than 110 % of the stated amount of Sevelamer Carbonate.

1. Identification:

1.1 Phosphate binding (by UV): The color obtained with test solution corresponds to the color obtained with standard solution in the test of phosphate binding.

1.2 Carbonate test: Weight accurately and transfer about 100 mg of sample into a test tube. Add 10ml of water and shake well. Allow the solution to stand for 2 minute. Add 2ml of dilute Hydrochloric acid along the side of the test tube. Brisk effervescences show the presence of carbonate.

Tests:

2. Phosphate binding capacity:

Solution A (0.02 M Phosphate solution): Accurately weigh about 0.680 g of Potassium dihydrogen phosphate anhydrous in 250 ml volumetric flask. Dissolve and make up to the volume with purified water.

Solution B (Ammonium molybdate solution): Accurately weigh about 5.0 g of Ammonium hepta molybdate in 100 ml of volumetric flask. Dissolve it in 40.0 ml of purified water. To it carefully add 15.0 ml of concentrated sulphuric acid and allow it to cool at room temperature and make up to the volume with purified water.

Solution C (Quinol Solution): Accurately weigh about 1.25 g of Quinol (Hydroquinone) in 250 ml volumetric flask. Dissolve and make up to the volume with purified water.

Solution D (Sodium sulphite solution): Accurately weigh about 10.0 g of anhydrous sodium sulphite in 100 ml volumetric flask. Dissolve and make up to the volume with purified water.

Blank solution: Transfer 1.0 ml concentrated Hydrochloride acid, 2.5 ml of **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** into 25 ml of volumetric flask and make up to the volume with purified water. Keep this solution aside for 30 minutes.

Standard solution (stock solution): Transfer 5.0 ml of **Solution A** in 250 ml of volumetric flask and make up to the volume with purified water. Use this solution for the preparation of calibration plot solutions.

Solution for calibration plots:

Solution 1 (3.2 X 10^{-5} mole): Transfer 2.0 ml of **stock solution** into 25 ml volumetric flask. Add to it 1.0 ml concentrated hydrochloric acid, 2.5 ml of **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** and make up to the volume with purified water. Keep this solution aside for 30 minutes. After 30 minutes measure the absorbance of resulting solution at 800 nm against blank solution.

Solution 2 (6.4 X 10^{-5} mole): Transfer 4.0 ml of **stock solution** into 25 ml volumetric flask. Add to it 1.0 ml concentrated hydrochloric acid, 2.5 ml of **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** and make up to the volume with purified water. Keep this solution aside for 30 minutes. After 30 minutes measure the absorbance of resulting solution at 800 nm against blank solution.

Solution 3 (9.6 X 10^{-5} mole): Transfer 6.0 ml of **stock solution** into 25 ml volumetric flask. Add to it 1.0 ml concentrated hydrochloric acid, 2.5 ml of **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** and make up to the volume with purified water. Keep this solution aside for 30 minutes. After 30 minutes measure the absorbance of resulting solution at 800 nm against blank solution.

Solution 4 (12.8 X 10^{-5} mole): Transfer 8.0 ml of **stock solution** into 25 ml volumetric flask. Add to it 1.0 ml concentrated hydrochloric acid, 2.5 ml of **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** and make up to the volume with purified water. Keep this solution aside for 30 minutes. After 30 minutes measure the absorbance of resulting solution at 800 nm against blank solution.

Solution 5 (16.0 X 10^{-5} mole): Transfer 10.0 ml of stock solution into 25 ml volumetric flask. Add to it 1.0 ml concentrated hydrochloric acid, 2.5 ml of **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** and make up to the volume with purified water. Keep this solution aside for 30 minutes. After 30 minutes measure the absorbance of resulting solution at 800 nm against blank solution.

Test Preparation: Accurately weigh about 100 mg eq. of sample in 250ml stoppered conical flask, to it add 50.0 ml of **solution A** (**0.02M Phosphate Solution**), adjust the pH of this solution to 7.0 by using dilute sodium hydroxide. Stir the solution vigorously for about 3 hours. After 3 hours check the pH of solution, if it is dropped or increased then adjust it to 7.0 by using dilute sodium hydroxide or dilute hydroxide acid. Filter the solution through whatman filter paper.

Transfer 10.0 ml of this filtrate to 250 ml of volumetric flask, make up the volume with purified water. (This is sample stock solution for color development).

Pipette out 10.0 ml of sample stock solution in to 25 ml of volumetric flask, to it add 1.0 ml of concentrated hydrochloric acid, 2.5 ml **solution B**, 2.5 ml of **solution C** and 2.5 ml of **solution D** and make up to the volume with purified water. Keep this solution aside for 30 minutes. After 30 minutes measure the absorbance of resulting solution at 800 nm against blank solution.

Calibration plot:

Plot the graph of concentration of phosphate (X-axis) against the absorbance at 800 nm (Y-axis). Draw the straight line.

Calculations:

Take 0.68 gm of potassium dihydrogen orthophosphate and dilute it to 250.0 ml (i.e; 0.02M solution A), from this transfer 50.0 ml solutions for analysis.

50 ml of 0.02 M of solution A = 0.095 gm of PO_4 (I)

One mole KH2PO4 = 1 mole of PO4

136.1 gm of KH2PO4 = 95 gm of PO4

Sample dilution = $\frac{0.1\ (\text{Eq.sample weight})}{50} \times \frac{10}{250} \times \frac{10}{25}$

Find out the molar phosphate concentration from the graph and calculate the total phosphate content in filtrate by following equation

Phosphate value derived from the plot = (**Sample Absorbance** + **Intercept**)/**Slope**

Molar phosphate PO4 concentration in filtrate (J)

= Phosphate concentration derived from the plot x 25 x 2.5

Where, 25 and 2.5 are the dilution factor shown in sample dilution

Find out the gm PO4 (phosphate) in filtrate by the following equation.

50 ml 0.02M of **solution A** = 0.095 gm of PO4

K (in gm) = (0.095 x J)/0.02

PO4 bound to sample in gram (L) = (I) – (K)

PO4 bound to sample in mg/tab (M) = L x avg. wt. / wt. of sample taken in gm

M mol of phosphate bound to per tablet = (M) / 95

Limit:

1.88 to 2.56 mmol/tablet

3. Assay of Sevelamer carbonate:

Sevelamer carbonate per tablet = (Phosphate binding capacity per Tab / 0.005) = ….. **mg/tab**

(**0.005m mole PO4 bonding contain 1.0mg Sevelamer Carbonate**)

% age of Sevelamer Carbonate = (mg/tab x 100)/label claim

4. Other tests: As per pharmacopoeial requirements.