

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: Weigh 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.

2. Phosphate binding capacity:

Solution A (0.02M Phosphate solution): Accurately weigh about 0.680 g of Potassium dihydrogen phosphate anhydrous in 250ml 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 100ml of volumetric flask. Dissolve it in 40.0ml of purified water. To it carefully add 15.0ml 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 250ml 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 100ml volumetric flask. Dissolve and make up to the volume with purified water.

Blank solution: Transfer 1.0ml concentrated Hydrochloride acid, 2.5ml of **solution B**, 2.5ml of **solution C** and 2.5ml of **solution D** into 25ml 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 250ml of volumetric flask and make up to the volume with purified water. Use this solution for the preparation of calibration plot solutions.

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Solution for calibration plots:

Solution 1 (3.2×10^{-5} mole): Transfer 2.0ml of **stock solution** into 25ml volumetric flask. Add to it 1.0ml concentrated hydrochloric acid, 2.5ml of **solution B**, 2.5ml of **solution C** and 2.5ml 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 800nm against blank solution.

Solution 2 (6.4×10^{-5} mole): Transfer 4.0ml of **stock solution** into 25ml volumetric flask. Add to it 1.0ml concentrated hydrochloric acid, 2.5ml of **solution B**, 2.5ml of **solution C** and 2.5ml 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 800nm against blank solution.

Solution 3 (9.6×10^{-5} mole): Transfer 6.0ml of **stock solution** into 25ml volumetric flask. Add to it 1.0ml concentrated hydrochloric acid, 2.5ml of **solution B**, 2.5ml of **solution C** and 2.5ml 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 800nm against blank solution.

Solution 4 (12.8×10^{-5} mole): Transfer 8.0ml of **stock solution** into 25ml volumetric flask. Add to it 1.0ml concentrated hydrochloric acid, 2.5ml of **solution B**, 2.5ml of **solution C** and 2.5ml 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 800nm against blank solution.

Solution 5 (16.0×10^{-5} mole): Transfer 10.0ml of stock solution into 25ml volumetric flask. Add to it 1.0ml concentrated hydrochloric acid, 2.5ml of **solution B**, 2.5ml of **solution C** and 2.5ml 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 800nm against blank solution.

Test Preparation: Accurately weigh about 100 mg eq. of sample in 250ml stoppered conical flask, to it add 50.0ml 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 hydrochloric acid. Filter the solution through whatman filter paper.

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

Pipette out 10.0ml of sample stock solution in to 25ml of volumetric flask, to it add 1.0ml of concentrated hydrochloric acid, 2.5ml **solution B**, 2.5ml of **solution C** and 2.5ml 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 250.0ml (i.e; 0.02M solution A), from this transfer 50.0ml solutions for analysis.

50ml of 0.02M of solution A = 0.095 gm of PO₄ (I)

One mole KH₂PO₄ = 1 mole of PO₄

136.1 gm of KH₂PO₄ = 95 gm of PO₄

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

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)

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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.