Dissolution test Method and HPLC analysis for Pantoprazole Pellets dosage forms.

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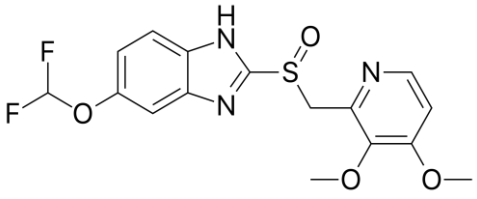
ABSTRACT: Pantoprazole belongs to benzimidazole group. It is a proton pump inhibitor, used for the treatment of gastric and duodenum ulcers. It undergoes degradation in acid medium of the stomach, can be coated with enteric coating polymer that will safely deliver the drug in the small intestine. A simple dissolution test method for Pantoprazole pellets dosage forms has been proposed and HPLC analytical method was developed for the measurement of Pantoprazole pellets dosage forms with Detector .The chromatographic system consists of 4.0mm x 12.5CM, 5µ Packing L7 column, an gradient mobile phase of 340 ml of Acetonitrile+660ml phosphate buffer. The flow rate is 1ml /minute and elute is tested at 280nm.The pantoprazole was eluted 4.569min with no interfering peak from additives used for preparation dosage forms. The method was linear over the range of 10-125µg/ml pantoprazole. The dissolution test was conducted in 6.8 phosphate buffer, 900ml with paddle RPM at 100.Dissolution was found to be NTL 75% in 45 minutes. The proposed method was applied successfully for the measurement of Pantoprozole content in pellets dosage forms

Key words: Dissolution tester, HPLC, Pantoprazole Pellets dosage forms, RPM, Mobile phase

1. INTRODUCTION: IUPAC name of pantoprazole is "6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzimidazole".It is a substituted benzimidazole specified for the short-term treatment (up to 16 weeks) in the healing and symptomatic relief of erosive esophagitis. Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production[1], it is due to lipophilic weak base that crosses the parietal cell membrane and enters the acidic parietal cell canaliculus where it becomes protonated, producing the active metabolite sulphenamide, which forms an irreversible covalent bond with two sites of the H+/K+-ATPase enzyme located on the gastric parietal cell, thereby inhibiting both basal and stimulated gastric acid production[2].

Structure of Pantoprazole:



2. Materials and Methods:

2.1 Instruments: Dissolution tester make Electrolab India (p)L.t.d, High performance liquid chromatograph shimadzu 2010 Rheoodyne injector with 100µl loop.L.C solutions computer based software is used.

2.2.Chemicals: Working standard pantoprazole procured from M/S Angels Pharma India Private Limited, Acetonitrile, HPLC grade(make E-Merck) ,Methylene chloride, Methanol (HPLC GRADE), Ammonia, Isopropyl alcohol(HPLC GRADE), Ethanol AR, Disodium tetraborate AR, Dibasic Sodium phosphate AR, Hydrochloric acid AR, Sodium Hydroxide AR, Monobasic sodium phosphate AR, Potassium dihydrogen orthophosphate and purified water.

2.3. Drug release

Acid resistance stage:

Medium	: 0.1 N Hydrochloric acid; 500mL
Apparatus	: USP-II
Rpm	:100
Time	: 2 hours

Buffer stage

Medium	: 6.8 phosphate buffer, 900ml
Apparatus	: USP-II
Rpm	: 100
Time	: 30min.

2.4**Requirements:**

Instruments: A suitable High Performance Liquid Chromatography consisting of a pump, a UV-VIS detector, sample injector, controller and integrator or equivalent software. The system is equipped with 4.6mm x 250mm, Inertsil ODS 3V or Equivalent. Dissolution apparatus, pH meter

Reagents; Methylene chloride, Methanol (HLC GRADE), Ammonia, Isopropyl alcohol(HLC GRADE), Ethanol AR, Disodium tetra borate AR, Dibasic Sodium phosphate AR, Hydrochloric acid AR, Sodium Hydroxide AR, Monobasic sodium phosphate AR, Acetonitrile (HLC GRADE), Potassium di hydrogen orthophosphate and purified water.

2.5 Solution Preparation:

Preparation of 0.01M of Disodium borate:
Weigh about 3.84g of Disodium tetraborate and make up to 100mL using wate, mix well.
0.1NHCI Preparation:
Take about 8.5ml of Hydrochloric acid and make up to 1000ml using water, mix well.

2N HCI Preparation:
Take about 17.0ml of Hydrochloric acid and make up to 100ml using water, mix well

2N NaOH Preparation
Weigh about 8.0g of NaOH and make up to 100ml using water, mix well.

pH 10.4 Buffer preparation:
0.235 M Dibasic sodium phosphate-Dissolve 33.36g of anhydrous sodium phosphate in 100ml of water. And adjust with 2N sodium Hydroxide to pH of 10 .4 $\pm$ 0.1

pH 6.8 Phosphate buffer preparation:

Add 400ml of 0.1N hydrochloric acid to 320 ml of pH 10.4, 0.235 M Dibasic sodium phosphate and adjust with 2N Hydrochloric acid or 2N sodium hydroxide, if necessary, to a pH of 6.8±0.05. Dilute 250ml of this solution with water to 1000ml.

pH 7.6 Phosphate Buffer preparation:

Dissolve 0.178g of monobasic sodium phosphate and 4.49g of dibasic sodium phosphate in 1000ml of water. Adjust with 2N Hydrochloric acid or 2N sodium hydroxide, if necessary, to a pH 7.6±0.1. Dilute 250ml of this solution with water to 1000ml.

Mobile phase preparation:

Transfer 340 ml of Acetonitrile to a 1000ml volumetric flask, dilute with pH 7.6 phosphate buffer to volume, and pass through a membrane filter having a 0.5um or finer porosity,. Make adjustments.

Diluents:-

Dissolve 7.6g of sodium borate dehydrates in about 800ml of water. Add 1.0g of edentate Disodium, and adjust with 50% sodium hydroxide solution to a pH of 11.0±0.1. Transfer the solution to a 2000ml volumetric flask, add 400ml of dehydrated alcohol, and dilute with water to volume.

Solution A- Prepare a filtered and degassed solution of 6.0g of glycogen in 1500ml of water. Adjust with 50% sodium hydroxide solution to a pH of 9.0, and dilute water to 2000ml.

Solution B- Use a filtered and degassed mixture of acetonitrile and methanol(85:15)

Mobile phase – Use variable mixture of solution A and solution B as directed for chromatographic system. Make adjustments if necessary.

3.0 Acid Stage Dissolution solutions preparation:

Standard solution:

Accurately weigh about 50 mg of Pantoprazole WS, in to a 250ml volumetric flask, dissolve in 50 ml of alcohol, dilute with 0.01M sodium borate solution to volume and mix. Transfer 10.0ml of this solution into a 100ml volumetric flask, add 20ml of alcohol, dilute with 0.01M sodium borate solution to volume and mix well.

Test solution:

Transfer 500ml 0.1N HCI solution in dissolution jar maintains the temp. $37.0^0C \pm 0.5^0C$ and accurately weigh 235.0mg of sample (pellets) transfer in to the jar. Start the dissolutions system. After 2 hours, filter the dissolution medium containing the pellets on the sieve, and rinse them aperture of not more than 0.2mm. after 2 hrs collect the pellets on the sieve, and rinse them with water.

Using approximately 60ml of 0.01M sodium borate solution, carefully transfer the pellets quantitatively to a 100ml volumetric flask. Sonicate for about 20minutes until the pellets are broken up. Add 20ml of alcohol to the flask, dilute with 0.01M sodium borate solution to volume, and mix. Dilute the an appropriate amount of this solution with 0.01M sodium borate at about 3000rpm for 5 minutes. Final Concentration 10µg/ml.

4.0 Buffer stage:

Medium: pH 6.8 phosphate buffer, 900ml

Proceed as directed for acid resistance stage with a new set of pellets from the same batch. After 2 hours, add 400 ml of 0.235M dibasic sodium phosphate to the 500ml of 0.1N hydrochloric acid medium in the vessel. Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH 6.8 ±0.05.

ApparatusUSP-II : 100rpm
At the end of 30 minutes, determine the amount of Pantoprazole dissolved in pH 6.8 phosphate buffer by employing the following method.

Dissolution Calculation:

$$\frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{std dilution}}{\text{sample dilution}} \times \frac{100}{Assay} \times \text{Stand purity} = \quad \text{---------}\%$$

5.0. Chromatographic parameters:

Column	: 4.0mm x 12.5CM, 5 µ Packing L7
Detector	: UV-Vis Detector
Wave length	: 280nm
Flow rate	: 1.0ml/minute
Injection volume	: 20 µL
Run time	: 12 minutes

Time (Minutes)	solution A %	solution B %	Elution
0-20	88-40	12-60	linear gradient
20-21	40-88	60-12	linear gradient
21-25	88	12	isocratic

System suitability:

Inject blank and record the chromatogram. Examine the chromatogram for any extraneous peaks. There should be no interference from the blank at the retention time of analyte peak. Inject standard solution five times and calculate the relative standard deviation (RSD) for area of the analyte peak. The relative standard deviation for five injections of Pantoprazole should not be more than 2.0%.

Separately: Inject equal volume (about 20µL) of the standard preparation and the test preparation into the chromatograph, record the chromatograms, and measure the peak area response. Calculate the quantity, in mg of Pantoprazole by the given formula

Dissolution Calculation:

$$\frac{\text{sample area}}{\text{Std area}} \times \frac{\text{std Dilution}}{\text{sample dilution}} \times \frac{100}{\text{Assay}} \times \text{std purity} = \_\_\_\_\_\%$$

6. Assay:

6.1 Requirements:

Instrument: A suitable high performance Liquid chromatograph consisting of a pump, a UV-VIS detector, sample injector, controller and integrator or equivalent software. The system is equipped with 4.6mm x 250mm, inertsil ODS 3V or Equivalent, Dissolution apparatus and pH Meter.

Reagents: Methanol (HPLC GRADE), Phosphorouspentixide, ammonia, hydrochloric acid AR, sodium hydroxide AR, Monobasic sodiumphospahte, Acetonitrile, (HPLC GRADE) and purified water.

6.2 pH 7.6 Phosphate Buffer preparation:
Dissolve 0.718g of monobasic sodium phosphate and 4.49g of dibasic sodium phosphate in 1000ml of water. Adjust with 2N Hydrochloric acid or 2N sodium hydroxide, if necessary, to a pH of 7.6± 0.1. Dilute 250ml of this solution with water to 100ml.

6.3 Mobile phase preparation:
Transfer 340ml of acetonitrile to a 1000ml volumetric flask, dilute with pH 7.6 phosphate buffer to volume, and pass through a membrane filter having a 0.5um or finer porosity. Make adjustments.

Diluent: Dissolve 7.6 g of sodium borate dehydrates in about 800ml of water. Add 1.0g of edentate Disodium, and adjust with 50% sodium hydroxide solution to a pH of 11.0±0.1. Transfer the solution to a 2000ml volumetric flask, add 400ml of dehydrated alcohol, and dilute with water to volume.

Blank solution: Take mobile phase as blank.

6.4 Chromatographic parameters:

Column	: 4.0mm x 12.5CM, 5µ Packing L7
Detector	: UV-Vis Detector
Wave length	: 280nm
Flow rate	: 1.0ml/minute
Injection volume	: 20µL
Run time	: 12 minutes

Procedure:

Time (Minutes)	solution A %	solution B %	Elution
0-20	88-40	12-60	Linear gradient
20-21	40-88	60-12	Linear gradient
21-25	88	12	isocratic

Standard preparation- Dissolve, by sonicating an accurately weighed quantity of Pantoprazole WS in diluent, and quantitatively, and stepwise if necessary, with diluents to obtain a solution having known concentration of about 0.2mg per mL.

Sample preparation- Weigh and mix the contents, Transfer an accurately weighed portion of the mixture about 235mg of Pantoprazole, to a 100mL volumetric flask, add about 50ml of membrane filter having 0.45µm of fine porosity. (Note – Bubbles may form just before bringing the solution to volume. Add a few drops dehydrated alcohol to dissipate the bubbles if the persist for more than few minutes.)

Blank solution: Take mobile phase as blank.

Procedure:
Separately inject equal volume (about 20µl) of the standard preparation and the test preparation into the chromatograph, record the chromatograph, and measure the peak area responses. Calculate the quantity, in mg of Pantoprazole by the given formula

Assay calculation: $\frac{\text{sample area}}{\text{Std area}} \times \frac{\text{standard dilution}}{\text{sample dilution}} \times \text{std purity} = \_\_\_\_\_\%$

Recovery studies: To study the linearity, accuracy and precision of proposed method, recovery experiments were carried out known quantities of standard at two different levels were added to the pre analyzed sample, the recovery was estimated to be more than 99%.

Conclusion: The proposed method is simple rapid and no where involves use of complicated sample preparation. High percentage of recovery shows that the method is free from interference of the excipients used in the semi formulations. Therefore method can be useful in routine quality control analysis.

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