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# Method Development and Validation for Estimation of Pantoprazole in Pantoprazole Formulations by Using HPLC

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**ABSTRACT:**

A highly selective, sensitive and accurate HPLC method has been developed and validated for the estimation of pantoprazole sodium in Pantoprazole formulation. The baseline separation of the pantoprazole peaks was achieved with 0.1% Formic Acid in water and 0.1% Formic acid in acetonitrile at a flow rate of 0.5 mL/min on a Waters Acquity CSH C18 column (2.1x100mm id,1.7µm). The total chromatographic run time was 10.0 min and the pantoprazole was eluted at 6.415min and PDA detection at wavelength of 288nm . The method was proved to be accurate and precise at linearity range of 2 µg/mL to 20µg/mL with a correlation coefficient (r) of ≥0.998. The intra- and interday precision and accuracy values were found to be within the assay variability limits. The developed method was validated according to ICH guidelines. The validation showed that developed method was valid and reliable for determination of active substance in pantoprazole formulations.

**Key words: HPLC, Pantoprazole, ICH guidelines**

## Introduction:

Peptic ulcers are ulcers that form in the stomach or the upper part of the small intestine, called the duodenum. Peptic ulcers are actually very common. A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to Helico bacter pylori that colonizes the antral mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis), resulting in a defect in the regulation of gastrin production by that part of the stomach, and gastrin secretion can either be increased, or as in most cases, decreased, results in hypo/achlorhydria. Gastrin stimulates the production of gastric acid by parietal cells, and in H. pylori colonization responses to increased gastrin, the increase in acid can contribute to the erosion of the mucosa and therefore an ulcer forms. The role of proton pump inhibits in ulcers by Inhibition of gastric acid secretion has been the major means of treatment of acid-related diseases, such as peptic ulcers and gastroesophageal reflux disease (GERD). The first medicinal target to be identified was the histamine-2 receptor, the major, but not the only one, activating parietal cell receptor. The second medicinal target was the gastric acid pump, the gastric (H+ , K+ )-ATPase. Since proton transport by the gastric (H+ , K+ )-ATPase is the final step in acid secre tion, it was anticipated that drugs of this type would be more effective inhibitor of acid secretion.

Drugs of Proton pump inhibitors Pantoprazole, Omeprazole, Esomperazole, Rabeprazole and Lanoprazole. Pantoprazole, 5-(difluoromethoxy) -2-[[(3,4-dimethoxy-2-pyridinyl)methyl] sulfinyl] -1H-benzimidazole is an oral pharmace utically active compound having promising anti-ulcer activity and belongs to the class of 2-[[(2- pyridyl) methyl]sulfinyl]-1H-benzimidazoles. Molecular weight of pantoprazole was 432.4 and its empirical formula is $C_{16}H_{14}F_2N_3NaO_4S$ x1.5 $H_2O$.Pantoprazole sodium sesquihydrate is a white to off-white crystalline powder and is racemic. Pantoprazole has weakly basic and acidic properties.PNT is a freely soluble in water, very slightly soluble in phosphate buffer at pH.7.4 and practically insoluble in n-hexane. PNT is frequently used for the cure of erosion and ulceration of the esophagus caused by a gastr oesophageal reflux disease. It is pharmace utically formulated as gastro-resistant tablets containing 40 or 20 mg pantoprazole sodium sesquihydrate.

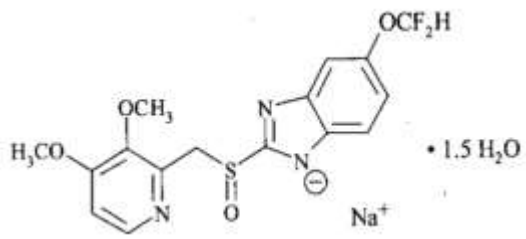


**Fig.1.Structure of Pantoprazole**

An extensive literature search revealed the retention times are long for Pantoprazole in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the simultaneous estimation of Pantoprazole in API and Pharmaceutical dosage form.

## Material and Methods:

**Standard:** Pantoprazole drug substance

**Chemicals:** Distilled water filtered with 0.22µm filters, Methanol, Acetonitrile, IPA, DMSO, DCM, n-Hexane, Ethyl acetate solvents and Formic acid reagents used, all are AR grade purchased from Merck (Mumbai, India)and Calibrated and sterilized glassware made by borosilic pvt.ltd.

Eppendrof tubes purchased from Tarsons India.

**Apparatus used:** Electronic Balance made by Shimadzu, Vertex Mixer made by Neuation Vertex Mixer, Sonicator MADE BY Branson 2800, Micro Pipette Brand Tranferpette,

HPLC made by Agilent India Pvt.Ltd.

**Instrumentation:**

An Agilent 1290 infinityII series LC system (Agilent Technologies, USA) consisting of an auto sampler , a quaternary pump, a diode array detector , a column compartment, degasser with operating software Chemstation openlabs was employed for HPLC analysis.

**Methodology:**

**Solubility of Pantoprazole:** weigh 1mg of standard in 6 different eppendrof tubes and see the solubility of pantoprazole and select suitable solvents.

**Preparation of Blank solution:** Diluent of preparation is used as blank solution for specificity determination. Before injecting into the system, the solution was filtered using a Millipore 0.45µm mdi filter.

**Preparation of standard stock solution:**

Standard solution was prepared by diluting 10mg of pantoprazole reference standard was dissolve in distilled water. This solution was then diluted with the distilled water to get final concentration of 1mg/mL.

**Preparation of working standard solution:**

1mL of above solution was pipette out and transfers to 10mL volumetric flask add 3mL distilled water and sonicate for 5min. Then add remaining distilled water to achieve concentration of 100µg/mL.

**Preparation of sample standard solution:**

From Pansa 40 Tablet total weigh 13mg and dissolve in 3mL of distilled water and sonicate for 5min.Then add remaining distilled water to achieve concentration of 1000µg/mL.

**Preparation of working standard solution:**

1mL of above solution was pipette out and transfers to 10mL volumetric flask add 3mL distilled water and sonicate for 5min. Then add remaining distilled water to achieve concentration of 100µg/mL.

**Optimization of Chromatographic Conditions:**

First trail with waters acquity CSH C18 column with water and Acentonitrile as mobile phase at a flow rate 0.7mL/min, column temperature is 25ºC,PDA detection at wavelength 260nm.

The peak was eluted but area, resolution, tailing and USP plate count was not satisfactory.

Second with waters acquity CSH C18 column with water and Acentonitrile as mobile phase at a flow rate 0.5mL/min, column temperature is 30ºC, PDA detection at wavelength 288nm. The peak was eluted resolution was not satisfactory.

After several trails chromatographic conditions were optimized as waters acquity CSH C18 column (2.1x 100mm id,1.7µm) with 0.1% Formic acid in water and Acentonitrile as mobile phase at a flow rate 0.5mL/min, column temperature is 35ºC,PDA detection at wavelength 288nm.At optimized conditions the peak area, resolution, tailing and USP plate count was good.

## Results and Discussion:

The experimental Results are discussed below with a brief procedure followed in the methodology

**Method development:**

The system was set up by equilibrating the column with mobile phase for 15 min at a flow rate of 0.5 mL/min and then injecting the solution. To determine the necessary parameters of the system, a single injection of blank and single injection pantoprazole(100 ppm) standard solution was performed .The height of the peak, resolution, and USP plate number were evaluated by using this optimized method.

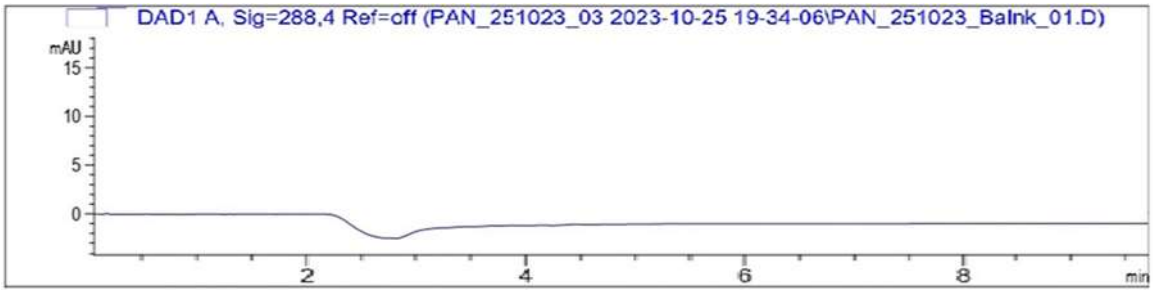


**Fig.2.Chromatogram of Blank**

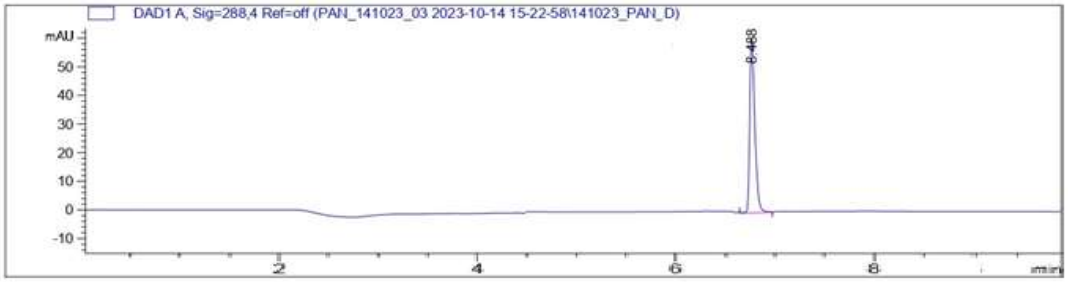


**Fig.3.Chromatogram of Pantoprazole Standard**

**Method Validation:**

The developed HPLC method was validated according to ICH guidelines like stability, linearity, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ), Robustness.

**1. System Suitability:** The system suitability parameters were determined by preparing standard solutions of pantoprazole 100ppm and the solutions were injected six times after injecting a blank solution and the parameters like peak tailing, resolution and USP plate count were determined.

**Table.1.System suitability Data of pantoprazole**

| Inj | RT(min) | USP Plate Count |
|---|---|---|
| 1 | 6.415 | 3874 |
| 2 | 6.420 | 3871 |
| 3 | 6.410 | 3978 |
| 4 | 6.416 | 3970 |
| 5 | 6.418 | 3969 |
| 6 | 6.413 | 3976 |

**Discussion:** According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters of pantoprazole were passed and were within the limits.

**2. Specificity:** Specificity of the method was determined by injecting blank and placebo to check whether peaks in the blank and placebo are interfering the elution of standard peaks.

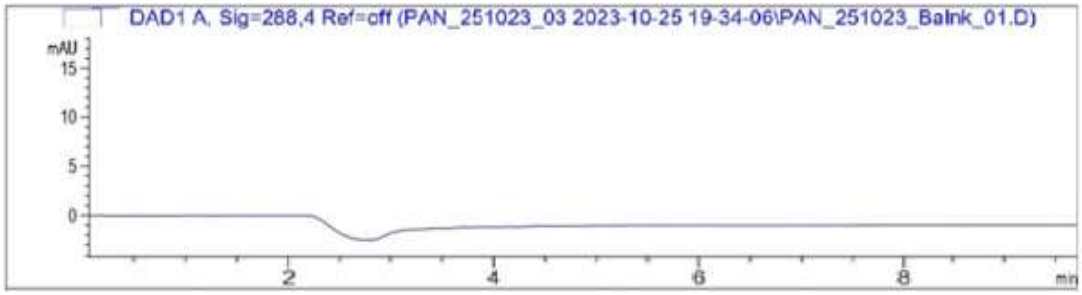


**Fig.4.Chromatogram of Blank**

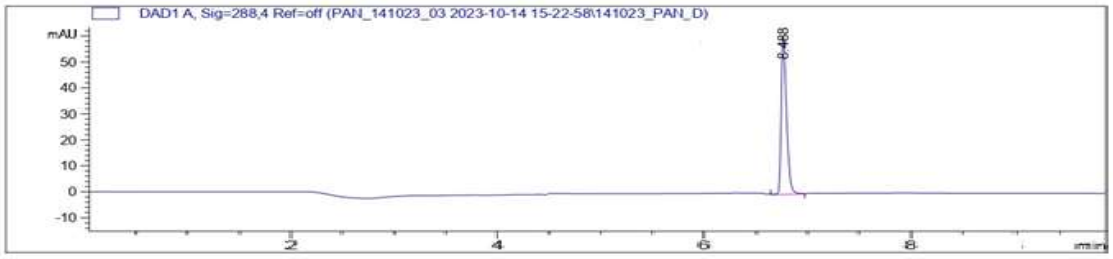


**Fig.5.Chromatogram of Pantoprazole Standard**

**Discussion:** Retention time of pantoprazole was 6.415.We did not found and interfering peaks in blank at retention times of these drugs in this method. So this method was said to be specific.

**3. Linearity:** Injected each level in to the chromatographic system and measured the peak area. Plotted a graph of peak area versus concen tration (on x-axis concentration and on y-axis peak area) and calculated the $R^2$.The linearity was determined by injecting the prepared concentratio n1ug/mL to 30ug/mL form working standard.

**Table.2. Linearity of Pantoprazole**

| Concentration | Peak Area |
|---|---|
| 1ppm | 30.21 |
| 5ppm | 155.22 |
| 10ppm | 323.73 |
| 15ppm | 495.07 |
| 30ppm | 915.076 |

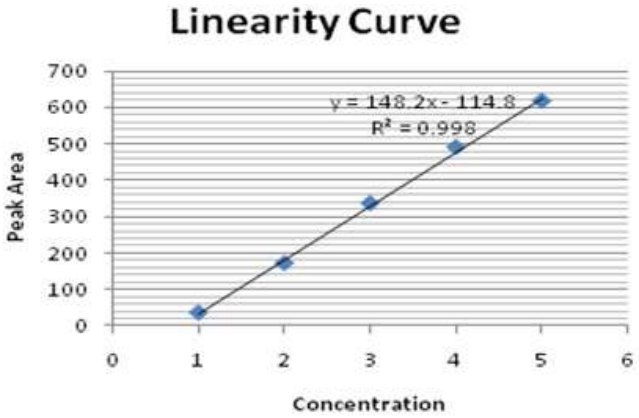


**Fig.6.Linearity Curve of Pantoprazole**

**Discussion:** The linearity of pantoprazole was determined was in the concentration range of 1 µg /mL-20 µg/mL with correlation of co-efficient $R^2$was found to be 0.998 with slope 148.2 and intercept was -114.8.The linearity of Pantoprazole were within the limits of ICH guideline.

**4. Precision:** Precision of an analytical method is the degree of agreement among individual test result when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Inject the blank one and standard for six times with 100ppm concentration in HPLC system. To determined the system precision, intraday (Same day analysis)and interday precision(Analysis on different days).

**Table.3. Precision Data of Pantoprazole**

| S. No | Method Precision | IntradayPrecision | Interday Precision |
|---|---|---|---|
| 1. | 1597390 | 1571771 | 1585385 |
| 2. | 1576291 | 1597975 | 1597119 |
| 3. | 1578947 | 1580273 | 1591827 |
| 4. | 1590840 | 1573913 | 1586648 |
| 5. | 1567675 | 1589010 | 1568641 |
| 6. | 1590354 | 1596167 | 1578939 |
| Mean | 1583583 | 1584852 | 1584760 |
| S.D | 11107.4 | 11218.5 | 10003.5 |
| %RSD | 0.7 | 0.7 | 0.6 |

**Discussion:** %RSD of pantoprazole for assay of six replicate preparations of method precision, intraday precision and interday precision sample s found to be 0.7,0.7 and 0.6 respectively. The precision was within the limits of ICH guidelines.

**5. Accuracy:** Accuracy or Recovery was to validate the closeness of test results obtained by the analytical procedure to the true value. The accuracy should be established across the specified range of the analyte concentration. Samples were prepared by spiking known amounts of the analyte at 50 to 150% which corresponds to 1 ppm to 20 ppm of target concentration of Pantoprazole API(about 15ppm) of the target assay to placebo.

**Table.4. Accuracy Data of Pantoprazole**

| % Level | Amount spiked | Amount recovered | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 7.5 | 22.2 | 100.8 | 100.93% |
| | 7.5 | 22.3 | 101.2 | |
| | 7.5 | 22.2 | 100.8 | |
| 100% | 15 | 30.3 | 101.0 | 100.20% |
| | 15 | 30.1 | 100.3 | |
| | 15 | 29.8 | 99.3 | |
| 120% | 18 | 33.2 | 100.5 | 100.03% |
| | 18 | 33.8 | 99.4 | |
| | 18 | 33.4 | 100.2 | |

**Discussion:** % Recovery of the pantoprazole was found to be 100.3%. The Accuracy was within the ICH limits guidelines.

**6. LOD and LOQ:** For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation and slope was adopted.

**Discussion:** The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve. The detection limit value was obtained as 0.25ppm and Quantitation limit was found to be 7.5ppm

**7. Robustness:** Small deliberate changes in method like flow rate, mobile phase ratio, and temperature were made. Robustness conditions like flow rate of +0.1mL/min, mobile phase ratio of + 5v/v, temperature +5◦C was maintained and samples were injected in triplicate manner.

**Table.5. Robustness Data of Pantoprazole.**

| Parameteres | Changes | Tailing Factor | USP Plate | %RSD |
|---|---|---|---|---|
| Flow rate | 0.3mL/min | 1.1 | 16351 | 0.1 |
| | 0.5mL/min | 1.1 | 11452 | 0.3 |
| | 0.7mL/min | 1.1 | 8039 | 0.3 |
| Column Temperature | 25ºC | 1.5 | 5183 | 0.3 |
| | 30ºC | 1.1 | 11452 | 0.3 |
| | 35ºC | 1.4 | 6304 | 0.3 |
| Mobile phase Composition | Low (90:10) | 1.8 | 15730 | 0.5 |
| | Organic(100 ACN+0.1% F.A) | 1.1 | 11452 | 0.3 |
| | High (ACN) | 1.2 | 8542 | 0.8 |
| Acceptance criteria | | NMT 2.0 | NLT 2000 | NMT 0.85 |

## Conclusion:

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

A simple, accurate, précised new HPLC method was developed for estimation of pantoprazole in pantoprazole formulation by trial and error method i.e, by using waters acquity CSH C18 column (2.1x100mm id, 1.7 µm) with mobile phase 0.1% formic acid in both water and acetonitrile, column temperature was maintained 35ºC, Injection volume 10µL and PDA detection wavelength at 288nm.Total run time of pantoprazole is 10mins.

The developed method was validated as per ICH guidelines. The develop method is suitability (table.no.1) and specificity for estimation of pantoprazole in pantoprazole formulations. The calibration curve of pantoprazole was obtained by plotting the respective peak area versus concentration range of 1ppm to 20ppm with correlation co-efficient ($R^2$) 0.998(table.no.2 and Fig.no.6). The %RSD of pantoprazole for precision method was found to be 0.7,0.7,and 0.6 for method precision, intraday precision and interday precision respectively(table.no.4.).The % recovery of the pantoprazole was found to be 100.3% (Table.no.4).The LOD and LOQ of the pantoprazole was found to be 0.25 ppm and 7ppm respectively. The %RSD for robustness of the pantoprazole was found within the limits (table.no.5).

Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity and hence it is suitable for routine analysis as well as for stability analysis.

**References**

1. **Paul w. Jungnickel;** pantoprazole: a new proton pump inhibitor; clinical therapeutics/vol. 22, no. 11, 2000

2. **Mukesh Kumar, Mohit Saini.,et al;** Preformulation Studies of Pantoprazole: Fundamental Part of Formulation Design; DOI: 10.36348/sjmps.2022.v08i08.001

3. B.k sharma, instrumental methods of chemical analysis, introduction to analytical chemistry, 23rd edition goel publication , meerut, (2007)

4. Lindholm.j, development and validation of hplc method for analytical and preparative purpose. Acta universitatis upsaliensis, pg . 13-14, (2004).

5. Rashmin, an introduction to analytical method development for pharmaceutical formulations. Indoglobal journal of pharmaceutical sciences , vol.2 , issue 2, pg 191-196 (2012).

6. Malvia r, bansal v , pal o.p and sharma p.k. A review of high performance liquid chromatography. Journal of global pharma technology (2010)

7. Gurdeep r.chatwal , sham k .anand, instrumental methods of chemical analysis , pg 2.566- 2.638 (2007)

8. David g. Watson pharmaceutical analysis, a text book for pharmacy students and pharmaceutical chemists. Harcourt publishers limited; 2nd ed.,pg-267-311**Pooja choudhry, kuldeep s patel.,,et al.** Design, in vitro evaluation and in vivo studies of novel delayed release tablets of pantoprazole. International journal of biomedical and advance research

9. **R ozgur karaca, said kalkisim**; effects of genetic polymorphisms of cytochrome p450 enzymes and mdr1 transporter on pantoprazole metabolism and helicobacter pylori eradication. Basic & clinical pharmacology & toxicology, 2017, 120, 199–206

10. **K. Karljikovic-rajic , d. Novovic .,et al**; first-order uv-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities; journal of pharmaceutical and biomedical analysis; 32 (2003) 1019 −/1027

11. **P. Ravi kumar , p. Bhanu prakash.,et al;** simultaneous estimation of domperidone and pantoprazole in solid dosage form by uv spectrophotometry; e-journal of chemistryvol. 3, no.3, pp 142-145, july 2006

12. **Asma rafiq, somia gul.,et al;** Quantitative analysis of pantoprazole sodium sesquihydrate in bulk and solid dosage form via uv-spectro