# CHAPTER-8

## Pantoprazole

**8.1** A survey of literature for Pantoprazole indicated the estimation reported by the following methods, Viz , High-performance liquid chromatography-electrospray ionization mass spectrometry, High-performance liquid chromatography , Capillary isotachophoresis.Gas chromatography-mass spectrometry, UV Spectrophotometric method , Micellar electrokinetic chromatography, Gas chromatography-mass spectrometry The brief information on above analytical methods are follows

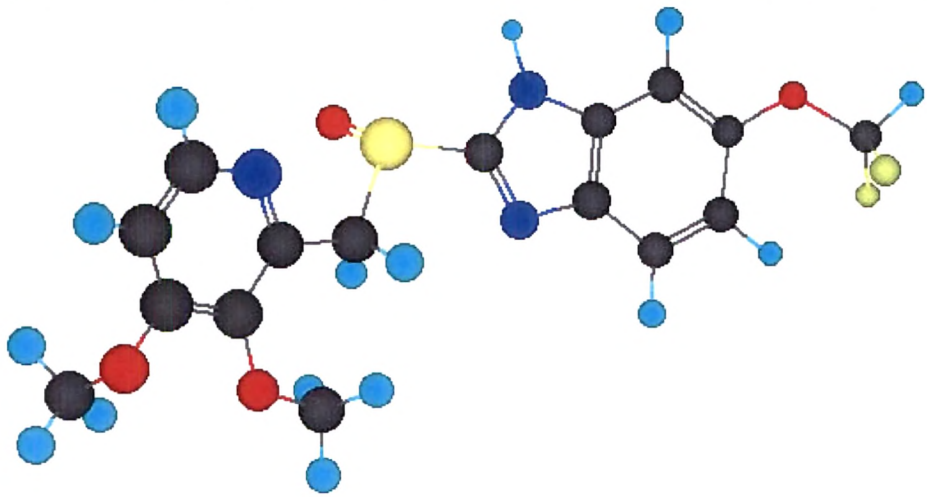
Moustafa A. et al [227] have reported Spectrophotometric for the determination of lansoprazole and pantoprazole sodium sesquihydrate. Ekpe A. et al [228 ] have reported Effect of various salts on the stability of lansoprazole, omeprazole, and pantoprazole as determined by high-performance liquid chromatography. Tivesten A. et al [229] have reported Nonaqueous capillary electrophoresis for the analysis of labile pharmaceutical compounds.Masubuchi N et al [230] have reported Stereoselective chiral inversion of pantoprazole enantiomers after separate doses to rats. Eberle D. et al [231] have reported Chiral resolution of pantoprazole sodium and related sulfoxides by complex formation with bovine serum albumin in capillary electrophoresis.

Tanaka M et al [232] have reported Direct determination of pantoprazole enantiomers in human serum by reversed-phase high-performance liquid chromatography using a cellulose-based chiral stationary phase and column-switching system as a sample clean up procedure.Tanaka M et al [233] have reported Direct HPLC separation of enantiomers of pantoprazole and other benzimidazole sulfoxides using cellulose-based chiral stationary phases in reversed-phase mode.Balmer K. et al [234] have reported Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases

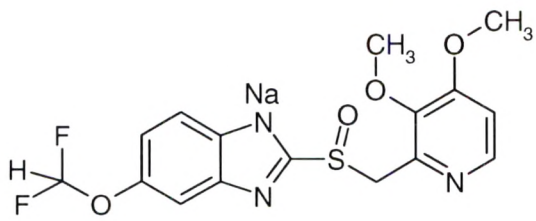
## 8.2 Analytical Method development of related substances determination in pantoprazole:

**INTRODUCTION :**

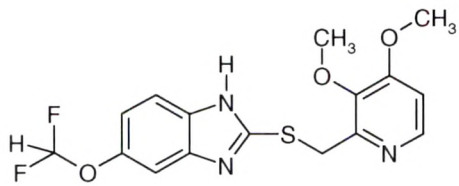
Pantoprazole sodium is a substituted benzimidazole, sodium 5-( difluoromethoxy)- 2- [[( 3,4- dimethoxy- 2- pyridinyl) methyl] sulfinyl]- 1 H- benzimidazole, compound that inhibits gastric acid secretion and indicated for the short- term treatment in the healing and symptomatic relief of erosive esophagitis. Pantoprazole sodium is a white to off-white crystalline powder, freely soluble in water and practically insoluble in n- hexane. Structures of pantoprazole sodium and various related impurities are described below.



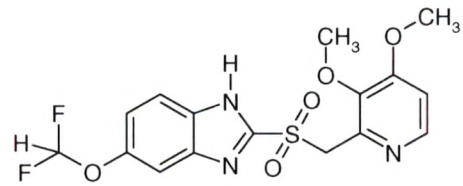
Pantoprazole sodium 3 D structure STR-20




Pantoprazole sodium STR-21

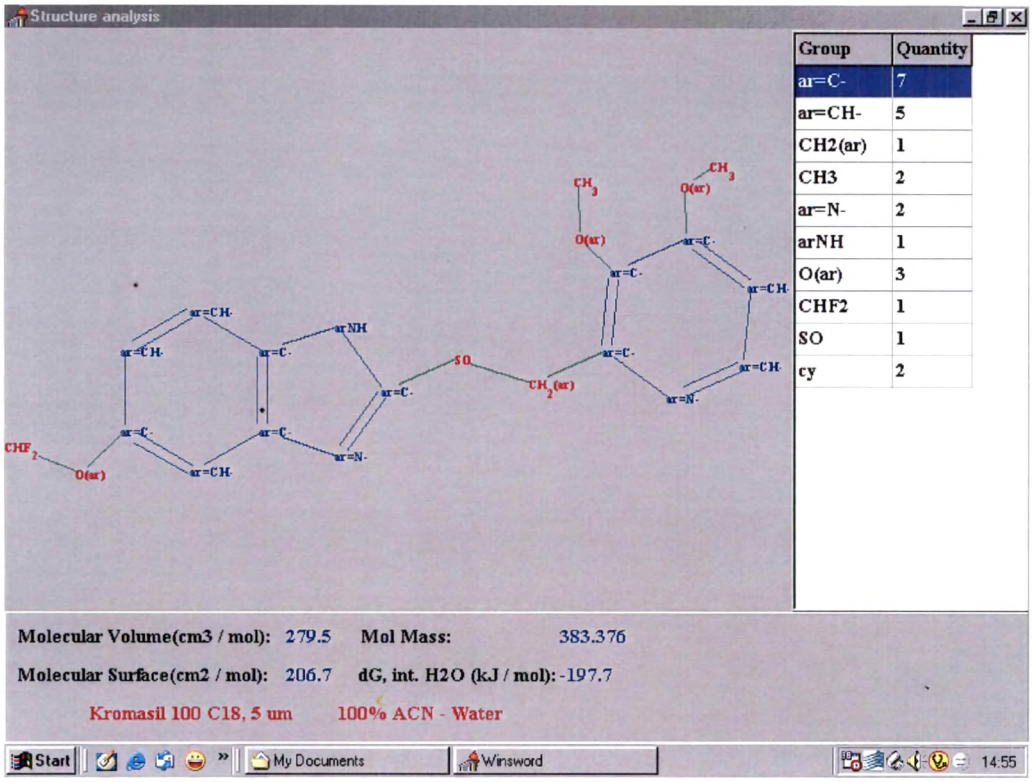



Impurity-A STR-22

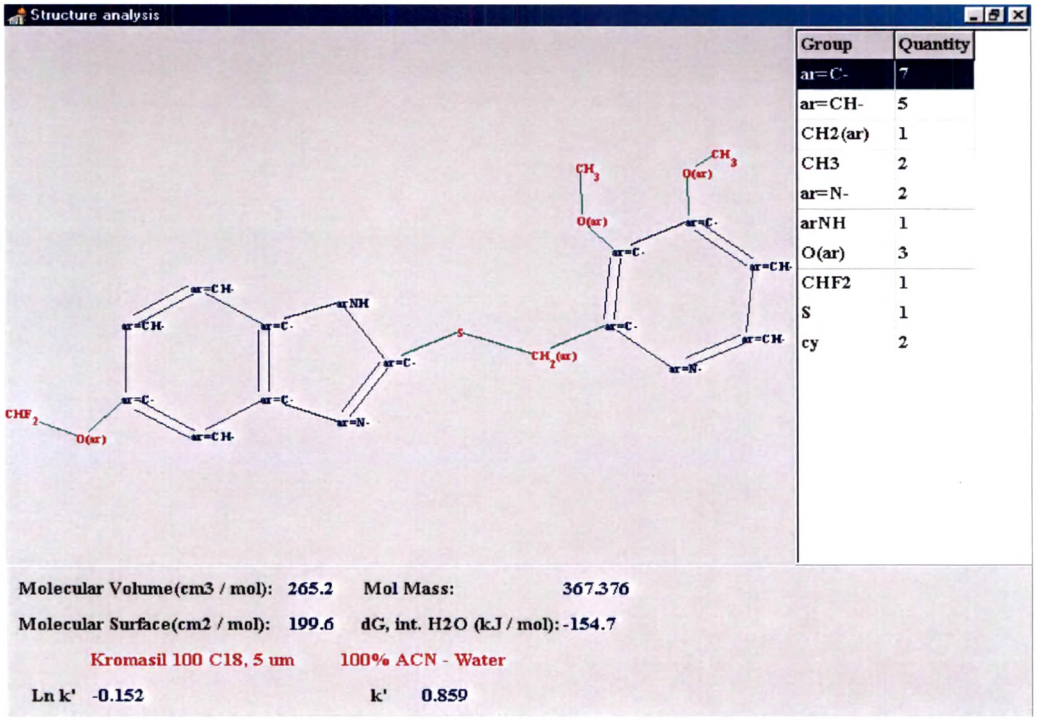



Impurity-B STR-23

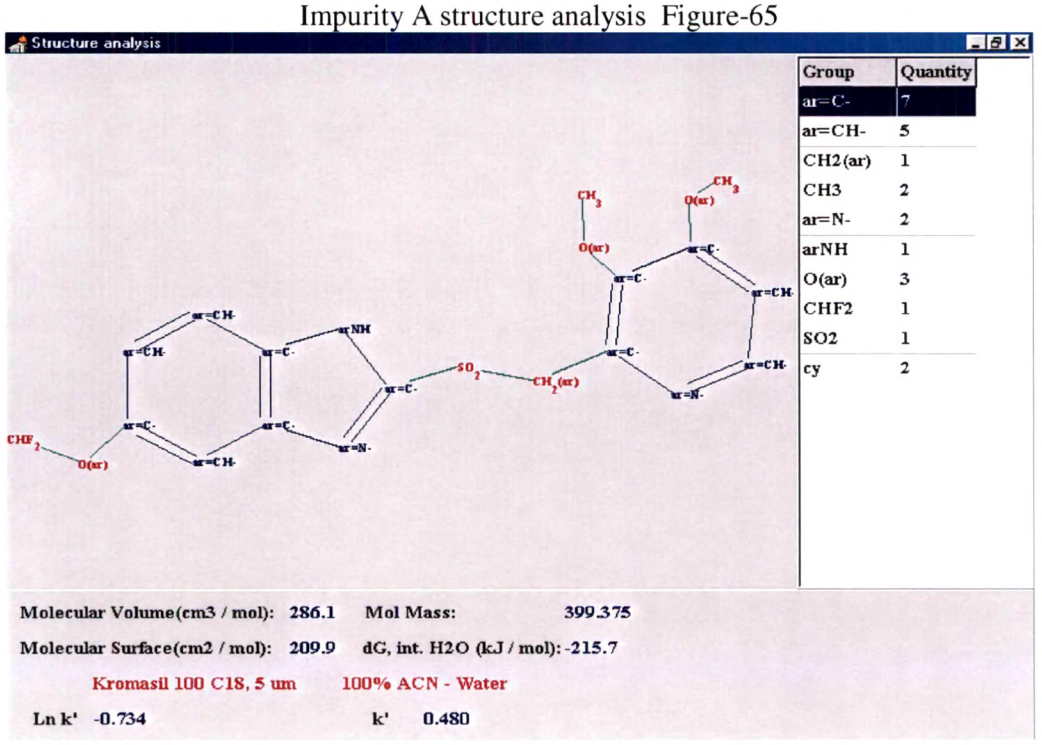
All structures are loaded into chromsword HPLC method development software to deduce structure analysis for method development. Figure-64 to 66 shows Pantoprazole and its related compounds structure analysis charts.




Pantoprazole structure analysis Figure-64

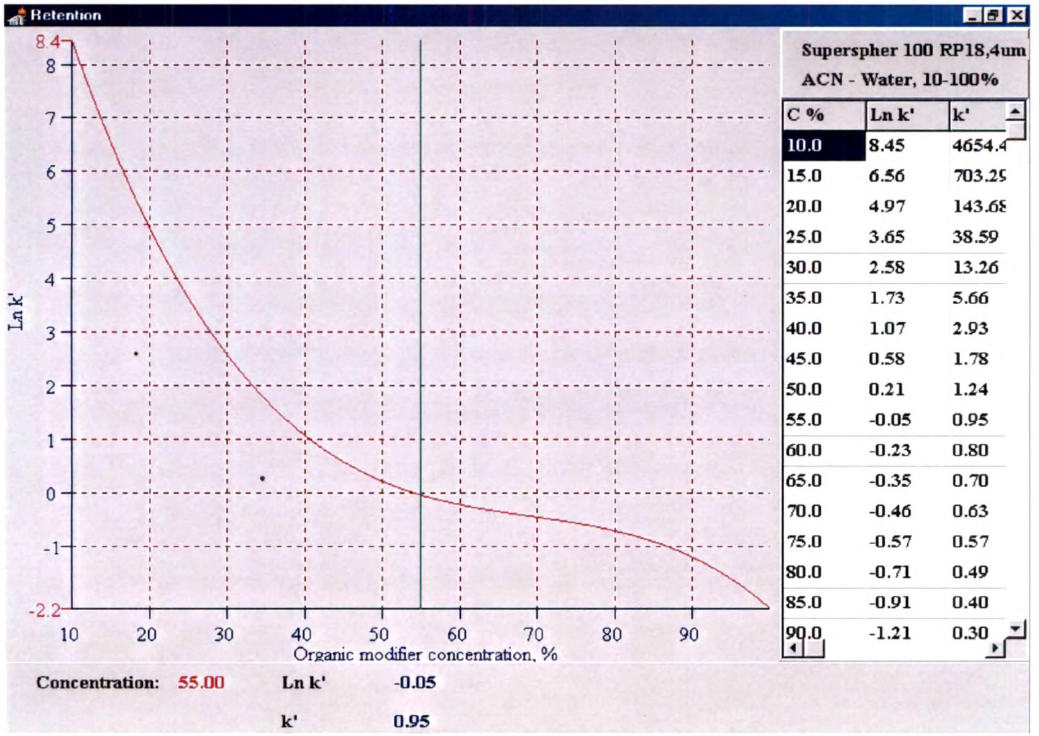



Impurity A structure analysis Figure-65



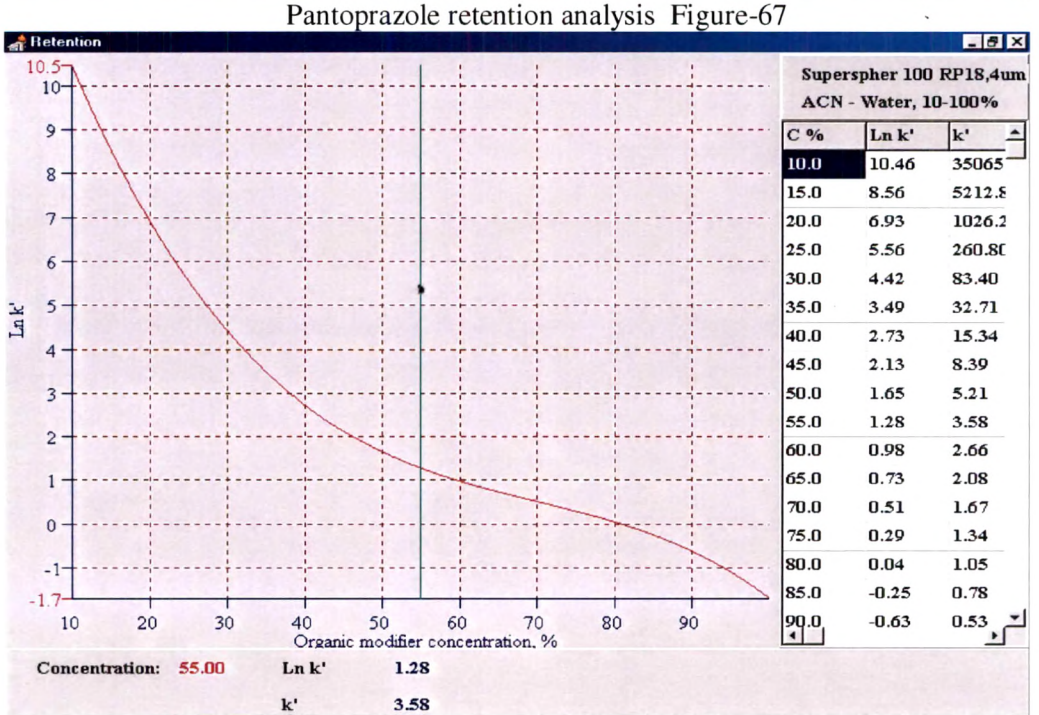

Impurity B structure analysis Figure-66

Figure-67 to 69 shows Pantoprazole and its related compounds retention analysis charts.



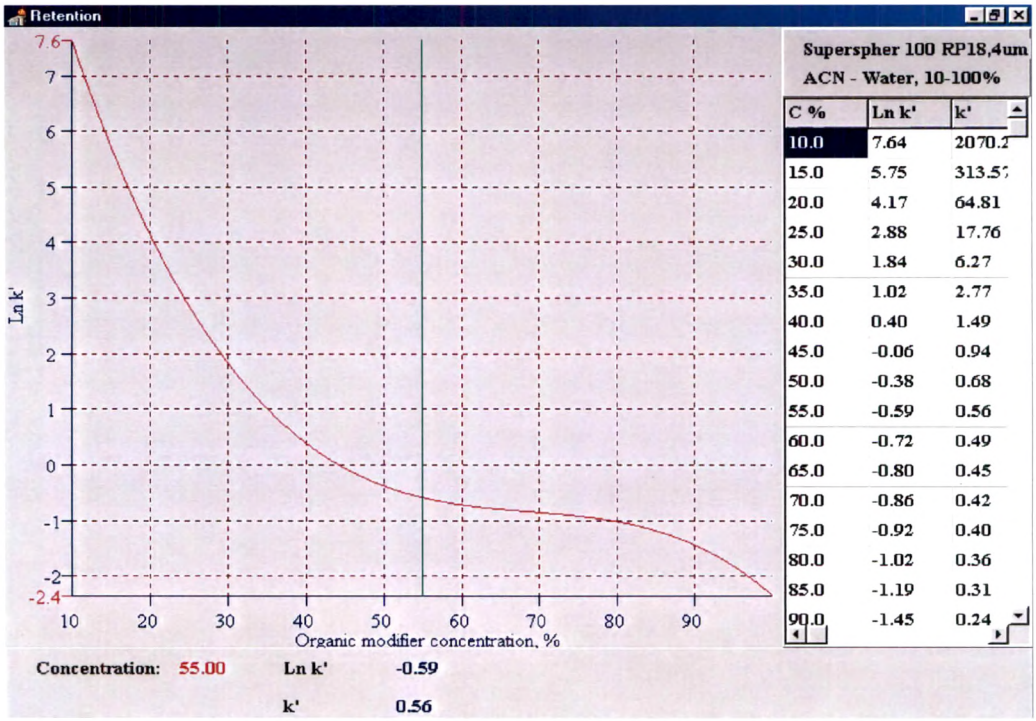

| Superspher 100 RP18,4um ACN - Water, 10-100% | | |
|---|---|---|
| C % | Ln k' | k' |
| 10.0 | 8.45 | 4654.4 |
| 15.0 | 6.56 | 703.25 |
| 20.0 | 4.97 | 143.65 |
| 25.0 | 3.65 | 38.59 |
| 30.0 | 2.58 | 13.26 |
| 35.0 | 1.73 | 5.66 |
| 40.0 | 1.07 | 2.93 |
| 45.0 | 0.58 | 1.78 |
| 50.0 | 0.21 | 1.24 |
| 55.0 | -0.05 | 0.95 |
| 60.0 | -0.23 | 0.80 |
| 65.0 | -0.35 | 0.70 |
| 70.0 | -0.46 | 0.63 |
| 75.0 | -0.57 | 0.57 |
| 80.0 | -0.71 | 0.49 |
| 85.0 | -0.91 | 0.40 |
| 90.0 | -1.21 | 0.30 |

Pantoprazole retention analysis Figure-67

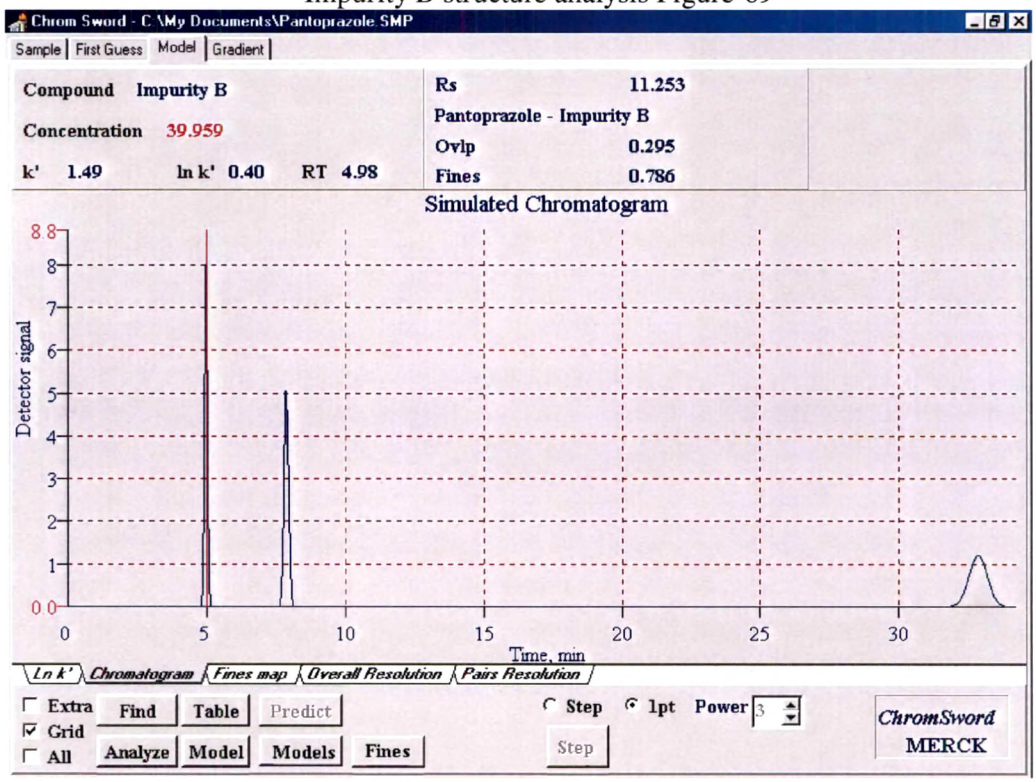



| Superspher 100 RP18,4um ACN - Water, 10-100% | | |
|---|---|---|
| C % | Ln k' | k' |
| 10.0 | 10.46 | 35065 |
| 15.0 | 8.56 | 5212.8 |
| 20.0 | 6.93 | 1026.2 |
| 25.0 | 5.56 | 260.8 |
| 30.0 | 4.42 | 83.40 |
| 35.0 | 3.49 | 32.71 |
| 40.0 | 2.73 | 15.34 |
| 45.0 | 2.13 | 8.39 |
| 50.0 | 1.65 | 5.21 |
| 55.0 | 1.28 | 3.58 |
| 60.0 | 0.98 | 2.66 |
| 65.0 | 0.73 | 2.08 |
| 70.0 | 0.51 | 1.67 |
| 75.0 | 0.29 | 1.34 |
| 80.0 | 0.04 | 1.05 |
| 85.0 | -0.25 | 0.78 |
| 90.0 | -0.63 | 0.53 |

Impurity A structure analysis Figure-68




Impurity B structure analysis Figure-69




Simulated chromatogram Figure-70

Figure-70 shows Pantoprazole and its related compounds simulated chromatograms

From the literature survey, it was found that pantoprazole sodium is not official in BP and USP and no method was reported for the analysis of related substances in pantoprazole sodium API. Therefore, it was thought to develop a new HPLC method for the analysis of related impurities in pantoprazole sodium API.

This report is describing different experimental trials carried out to develop a HPLC method for the analysis of related substances in pantoprazole sodium API. The different trials taken for the method development for the estimation of related substances are mentioned below.

**TRIAL –1**

Development of the method was started with an isocratic mobile phase composition comprised of volatile buffer. The chromatographic condition adopted in the trial-1 is as follows.

**Reagents :**

| | |
|---|---|
| Ammonium acetate | AR grade |
| Ammonia solution | AR grade |
| Methanol | HPLC grade |
| Water | HPLC grade |

**Mobile phase**: Buffer solution was prepared by dissolving 1.5 g of ammonium acetate in 1000 ml of water. pH of the solution was adjusted to 7.0 with ammonia solution. Mobile phase was prepared by mixing 500 ml of buffer solution and 500 ml of methanol. Mobile phase was filtered and degassed prior to use.

**Diluent :** Mobile phase

**Mixed solution of pantoprazole sodium and related substances :**

Accurately weighed 2 mg each of impurity A and impurity B were transferred in to a 100 ml of the volumetric flask. Dissolved the contents and diluted up to the mark with diluent. 1 ml of the above solution was transferred in to a 10 ml volumetric flask containing 10 mg of pantoprazole sodium. Dissolved the contents and diluted up to the mark with diluent

**Instrumental conditions :**

| | | |
|---|---|---|
| Column | : | Hypersil BDS C18 (250 x 4.6 mm), 5 µ |
| Flow rate | : | 1.5 ml/min. |
| Detection | : | 290 nm |
| Attenuation | : | Set appropriately |
| Run time | : | 40 minutes |
| Injection volume | : | 20 µl |

**Results and Discussion:**In the above chromatographic condition, impurity-A and impurity-B were well separated from the pantoprazole peak. However, an unknown impurity was eluted at the tail of pantoprazole peak. To separate the tailing impurity from pantoprazole, it was thought to modify the mobile phase composition.

**TRIAL-2:** From the previous trial, it was observed that an unknown impurity was eluted at the tail of pantoprazole peak. To separate the tailing impurity, mobile phase composition was slightly modified. The chromatographic condition adopted in trial-2 is as follows.

**Mobile phase**: Buffer solution was prepared by dissolving 1.5 g of ammonium acetate in 1000 ml of water pH of the solution was adjusted to 7.0 with ammonia solution. Mobile phase was prepared by mixing 600 ml of buffer solution and 400 ml of methanol. Mobile phase was filtered and degassed prior to use.

**Instrumental conditions:**

| | | |
|---|---|---|
| Column | : | Hypersil BDS C18 (250 x 4.6 mm), 5 µ |
| Flow rate | : | 1.5 ml/min. |
| Sample cooler temp | : | 4°C |
| Detection | : | 290 nm |
| Attenuation | : | Set appropriately |
| Run time | : | 65 minutes |
| Injection volume | : | 20 µl |

**Result and discussion:**

In the above trial, the tailing impurity as well as all other related impurities were well separated from pantoprazole peak. The above trial fulfilled the all requirements of the intended method i.e. identification and separation of all the related impurities. Finally this

method is recommended for the estimation of pantoprazole and its related substances in pantoprazole sodium API. Retention time and relative retention time of pantoprazole and its related substances are given table 1.

| Components | Retention time (min) | Relative retention time |
|---|---|---|
| Pantoprazole impurity B | 13.7 | 0.63 |
| Pantoprazole | 21.9 | 1.00 |
| Pantoprazole impurity A | 51.7 | 2.36 |

**8.3 Analytical Method:** Based on structure, retention analysis and trials performed in section 8.2 HPLC method is optimized and finalized is given below. Methanol is preferred over acetonitrile as methanol is found more suitable for resolution between impurities and better stability.

**Reagents and chemicals**

| | | | |
|---|---|---|---|
| 1) | Ammonium acetate | : | AR grade |
| 2) | Ammonia solution (25 % v/v) | : | AR grade |
| 3) | Methanol | : | HPLC grade |
| 4) | Water | : | HPLC grade |

**Buffer solution:** Dissolve 1 50 g of ammonium acetate in 1000 ml of water. Adjust the pH to 7.0 ±0.1 with 25 % (v/v) ammonia solution.

**Mobile phase: Prepare** mobile phase by mixing 60 volumes of buffer solution and 40 volumes of methanol. Filter and degas prior to use.

**System suitability preparation: Transfer** about 2.5 mg each of pantoprazole impurity B and pantoprazole sodium accurately weighed, to a 25 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase (100 µg/ml each of pantoprazole impurity B and pantoprazole sodium).

**Standard preparation:** Transfer about 5 mg each of pantoprazole impurity A, pantoprazole impurity B and 2 5 mg of pantoprazole accurately weighed, to a 100 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase. Transfer 2 ml of this solution to a 50 ml volumetric flask, mix and dilute it up to mark with mobile phase (2 µg/ml each of pantoprazole impurity A, pantoprazole impurity B and 1 µg/ml pantoprazole sodium).

**Sample preparation:** Transfer about 50 mg of pantoprazole sodium sample, accurately weighed, to a 50 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase (1000µg/ml).

**Instrumental conditions**

Use a suitable High Performance Liquid Chromatograph (HPLC) with the following conditions.

| | | |
|---|---|---|
| Column | : | Hypersil C18 BDS, 25cmx 4 6 mm, 5 µ, (Thermoquest, U.K) |
| Flow rate | : | 1.0 ml/min. |
| Detector | : | 290 nm |
| Attenuation | : | Set appropriately |
| Run time | : | About 70 mins |
| Injection volume | : | 20 µl |

**Procedure**

(1) Set up chromatographic system as described under instrumental conditions

(2) System suitability

Inject 20 µl of system suitability solution and calculate the resolution between pantoprazole impurity B and pantoprazole It should not be less than 10 The theoretical plates of pantoprazole peak should not be less than 3000 and tailing factor of pantoprazole should not be more than 2 0. The relative retention time (RRT) of pantoprazole impurity B and pantoprazole sodium are 1 and 2 respectively.

(3) Inject 20 µl standard solution six times and calculate the mean and RSD of area counts of injections. The relative standard deviation should not be more than 5.0%.

(4) Prepare samples and make injections and calculate the amount of related substances using the formula given in calculations.

**Calculations**

i) Calculate the percentage of pantoprazole impurity A, pantoprazole impurity B using the formula:

$$\frac{r_s}{r_{im}} \times \frac{C_{im}}{C_S} \times P$$

Where :

$C_{im}$ = Concentration of pantoprazole impurity A and pantoprazole impurity B in standard solution (µg/ml).

$C_S$ = Concentration of sample solution (µg/ml)

$r_s$ = Detector response for pantoprazole impurity A and pantoprazole impurity B in sample solution.

$r_{im}$ = Detector response for pantoprazole impurity A in standard solution.

P = Purity of pantoprazole impurity A and pantoprazole impurityB.

ii) Calculate the other impurities using the formula.

$$\frac{r_s}{r_{im}} \times \frac{C_{im}}{C_S} \times P$$

Where

$C_{im}$ = Concentration of pantoprazole sodium in standard solution (µg/ml)

$C_S$ = Concentration of sample solution (µg/ml).

$r_s$ = Detector response for any other impurity individually in sample solution

$r_{im}$ = Detector response for pantoprazole in standard solution.

P = Purity of pantoprazole sodium (as is basis).

**Limit of impurities**

| | | |
|---|---|---|
| 1) | Known impurity | : Not more than 0.2 % |
| 2) | Unknown impurity | Not more than 0 1 % |
| 3) | Total impurities | . Not more than 1.0 % |

## 8.4 Method validation

For this validation study following reagents, chemicals, equipments and chromatographic conditions were used for all experiments unless specified otherwise.

**a) Reagents and chemicals**

| | | | |
|---|---|---|---|
| 1) | Ammonium acetate | : | AR grade |
| 2) | Ammonia solution (25 % v/v) | : | AR grade |
| 3) | Methanol | : | HPLC grade |
| 4) | Water | : | HPLC grade |

**b) Working standard and sample**

**1) Pantoprazole sodium WRS**

B.No.1370/F/518/37 (Supplied by Sun Pharmaceuticals Industries limited.)

**2) Pantoprazole impurity A**

B No. Lot-1 (Supplied by Sun Pharmaceuticals Industries limited )

**3) Pantoprazole impurity B**

B.No 553/08 (Supplied by Sun Pharmaceuticals Industries limited )

**c) High performance liquid chromatograph**

- Shimadzu LC-10AT*vp* solvent delivery pump with SIL-10A*vp* Autoinjector.
- Shimadzu SPD-10Avp detector.
- Class VP data integrating software.

**d) System suitability parameters:** System suitability parameter i.e resolution was determined before any analysis was carried out

## 8.5 Identification

To determine the relative retention times of pantoprazole sodium pantoprazole impurity A and pantoprazole impurity B following solutions were prepared.

**Pantoprazole sodium** :1.016 mg of pantoprazole sodium WRS was weighed accurately and transferred to 10ml volumetric flask containing 5 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Pantoprazole impurity A** :1.011 mg of pantoprazole impurity A was weighed accurately and transferred to 10 ml volumetric flask containing 5 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase

**Pantoprazole impurity B** :1.017 mg of pantoprazole impurity B was weighed accurately and transferred to 10 ml volumetric flask containing 5 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

20 µl of mobile phase and each of the above solutions was injected into chromatographic system The relative retention times of both the components are given below in Table

| Components | Retention time (min) | Relative retention time |
|---|---|---|
| Pantoprazole impurity B | 13.6 | 0.63 |
| Pantoprazole | 21.3 | 1.00 |
| Pantoprazole impurity A | 51.3 | 2.41 |

Figure-146 shows typical chromatogram of mobile phase.

Figure-148 to Figure-150 shows typical chromatogram of pantoprazole, pantoprazole impurity A and pantoprazole impurity B individually for identification.

## 8.6 SYSTEM SUITABILITY

**System suitability preparation :**2 51 mg of pantoprazole impurity A and 2.54 mg of pantoprazole sodium WRS was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, and volume was made up with mobile phase mobile phase (100 µg/ml of pantoprazole sodium and 100 µg/ml pantoprazole impurity B)

20 µl of this solution was injected into the chromatographic system and the resolution factor was determined. The resolution obtained between pantoprazole sodium and pantoprazole impurity A peak was 18.20, theoretical plates of pantoprazole sodium peak was 9930 and tailing factor was 1.14

Figure-147 shows typical system suitability chromatogram.

## 8.7 INSTRUMENT PRECISION

**Stock solution**

**Solution A :**25.07 mg of pantoprazole impurity A was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution B** :25.08 mg of pantoprazole impurity B was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution C** :12.54 mg of pantoprazole sodium WRS was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution D** :5 ml each of solution A and solution B was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

**Solution E** :5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

**System precision solution preparation** : 2 ml each of solution D and solution E were transferred to 50 ml volumetric flask, mixed and diluted to volume with mobile phase.

System precision solution was injected six times, mean and RSD of detector response of pantoprazole sodium and pantoprazole impurity A was calculated. Table-8.7.1 shows the area counts of individual injection, mean and RSD.

**Table-8.7.1 Instrument precision**

| *Injection* | *Detector response (area counts)* | | | *Limits* |
|---|---|---|---|---|
| | *Pantoprazole impurity A* | *Pantoprazole impurity B* | *Pantoprazole* | |
| 1 | 62151 | 60768 | 33456 | |
| 2 | 63194 | 61462 | 35513 | |
| 3 | 64168 | 61166 | 35096 | |
| 4 | 63340 | 61174 | 33679 | |
| 5 | 64727 | 61374 | 33595 | |
| 6 | 65518 | 61397 | 35507 | |
| Mean | 63850 | 61224 | 34474 | |
| RSD (%) | 1.88 | 0 42 | 2.89 | NMT 5.0 % |

## 8.8 Specificity Of The Method

Specificity of the method was established by demonstrating no interference from degradation products. This was demonstrated by carrying out forced degradation of the sample by adding 5 ml each of 5M HCl, 5M NaOH and 30 % (v/v) $H_2O_2$, separately. The sample was also exposed under UV light for 24 hrs, and sunlight for 6 hr. The samples were prepared as given below and were injected into HPLC with a

Shimadzu SPD–10MAvp (photodiode array detector). The chromatograms were recorded upto 30 min. In each case % peak purity of pantoprazole sodium peak was determined to examine interference from degradation product and % of residual drug was calculated.

**Standard preparation**

50.11 mg of pantoprazole sodium standard was weighed accurately and transferred to 50 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made with mobile phase.

### 8.8.1 Acid degradation

50.07 mg pantoprazole sodium was transferred to a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this, 5 ml of 5M HCl was added. This solution was heated on boiling water bath for 10 min. After cooling, pH was adjusted to 7.0 with 5M NaOH and volume was made up with mobile phase. 5 ml of this solution was diluted to 10 ml with mobile phase and mixed thoroughly. This solution was injected into the HPLC and % residual drug was calculated by comparing area count with that of standard. Peak purity of pantoprazole sodium peak as determined by diode array detector was >99 %. Figure-155 shows HPLC chromatogram of acid degradation.

### 8.8.2 Alkali degradation

50.65 mg pantoprazole sodium was transferred to a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this, 5 ml of 5M NaOH was added. This solution was heated on boiling water bath for 10 min. After cooling, pH was adjusted to 7.0 with 2M HCl and volume was made up with mobile phase. 5 ml of this solution was diluted to 10 ml with mobile phase and mixed thoroughly. This solution was injected into the HPLC and % residual drug was calculated by comparing area count with that of standard. Peak purity of pantoprazole sodium peak determined by diode array detector was >99%. Figure-156 shows HPLC chromatogram of alkali degradation.

### 8.8.3 Peroxide degradation

50 11 mg pantoprazole sodium was transferred to a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this 5 ml of 30% $H_2O_2$ was added. This solution was

heated on boiling water bath for 10 min. After cooling the volume was made up with mobile phase. 5 ml of this solution was diluted to 10 ml with mobile phase and mixed thoroughly. This solution was injected into the HPLC and % residual drug was calculated. Peak purity of pantoprazole sodium peak as determined by diode array detector was >99%. Figure-157 shows HPLC chromatogram of peroxide degradation.

### 8.8.4 Degradation under UV light

50.07 mg pantoprazole sodium (previously exposed under UV light at 254 nm for 24 hrs) was transferred to a 50 ml volumetric flask containing 5 ml water, dissolved in and diluted to volume with mobile phase. 5 ml of this solution was diluted to 10 ml with mobile phase and mixed thoroughly. This solution was injected into the HPLC and % residual drug was calculated. Peak purity of pantoprazole sodium peak as determined by diode array detector was >99 %.

### 8.8.5 Degradation under Sun light

50.11 mg pantoprazole sodium (previously exposed under sun light for 6 hrs.) was transferred to a 50 ml volumetric flask containing 5 ml water, dissolved in and diluted to volume with mobile phase. 5 ml of this solution was diluted to 10 ml with mobile phase and mixed thoroughly. This solution was injected into the HPLC and % residual drug was calculated. Peak purity of pantoprazole sodium peak as determined by diode array detector was >99%. Figure-159 shows HPLC chromatogram for degradation under sunlight.

### 8.8.6 Thermal degradation

49.51 mg pantoprazole sodium was transferred to a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this 10 ml water was added. This solution was heated on a boiling water bath for 30 min. After cooling volume was made up with mobile phase. 5 ml of this solution was diluted to 25 ml with mobile phase and mixed thoroughly. This solution was injected into the HPLC and % residual drug was calculated. Peak purity of pantoprazole sodium peak as determined by diode array detector was >99%. Figure-158 shows HPLC
Chromatogram for thermal degradation .

## 8.9 Linearity Study

The linearity of detector (UV) response for pantoprazole impurity A, pantoprazole impurity B and pantoprazole sodium was determined by preparing and injecting solutions in the concentration range of 50 – 150 % of limit concentration. The solutions for linearity study were prepared as shown below.

**Solution A** :25 07 mg of pantoprazole impurity A was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase After sonication for 10 min, volume was made up with mobile phase.

**Solution B** :25.08 mg of pantoprazole impurity B was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution C** :12.54 mg of pantoprazole sodium WRS was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution D** :5 ml each of solution A and solution B was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

**Solution E** :5 ml of solution C was transferred in to a 100 ml volumetric flask Mixed and diluted to volume with mobile phase.

Using above solution following solutions were prepared as shown in Table-8 9 1.

**Table-8.9.1 : Solution preparation for linearity study of pantoprazole impurity A, pantoprazole impurity B and pantoprazole sodium**

| *% level of standard* | *Vol. of stock solution C (ml)* | *Vol. of stock solution D (ml)* | *Final dilution (ml)* | *Final Concentrations (µg/ml)* | | |
|---|---|---|---|---|---|---|
| | | | | *Pantoprazole impurity A* | *PantoprazoleImpurity B* | *Pantoprazole* |
| 50 | 1.0 | 1 0 | 50 0 | 1.00 | 1.00 | 0.50 |
| 75 | 1 5 | 1 5 | 50.0 | 1.50 | 1 50 | 0.75 |
| 100 | 2 0 | 2.0 | 50 0 | 2.00 | 2.00 | 1.00 |
| 125 | 2.5 | 2.5 | 50 0 | 2 50 | 2.50 | 1.26 |
| 150 | 3.0 | 3 0 | 50.0 | 3.00 | 3.01 | 1.51 |

20 µl of each of the above solutions was injected in triplicate into chromatographic system and mean area counts were calculated. Results of these analysis are shown in Table-8.9.2 to 8.9.4. **Table-8.9.2 : Linearity of pantoprazole impurity A**

| *% level of standard* | *Detector response (area counts)* | | | | *Limit* |
|---|---|---|---|---|---|
| | *Inj. 1* | *Inj. 2* | *Inj. 3* | *Mean* | |
| 50 | 43488 | 43775 | 43373 | 43545 | |
| 75 | 65179 | 64256 | 65171 | 64869 | |
| 100 | 87031 | 86644 | 86458 | 86711 | |
| 125 | 109619 | 109012 | 109112 | 109248 | |
| 150 | 131038 | 131333 | 131576 | 131316 | |
| Slope | | | | 43966 | |
| Intercept | | | | -830 | |
| Coefficient of correlation (r) | | | | 1.000 | NLT 0 99 |

**Table-8.9.3 : Linearity of pantoprazole impurity B**

| *% level of standard* | *Detector response (area counts)* | | | | *Limit* |
|---|---|---|---|---|---|
| | *Inj. 1* | *Inj. 2* | *Inj. 3* | *Mean* | |
| 50 | 29760 | 29855 | 29762 | 29792 | |
| 75 | 45007 | 45085 | 45062 | 45051 | |
| 100 | 60151 | 60264 | 60546 | 60320 | |
| 125 | 75652 | 75551 | 75790 | 75664 | |
| 150 | 91023 | 90845 | 90842 | 90903 | |
| Slope | | | | 30482 | |
| Intercept | | | | -788 | |
| Coefficient of correlation (r) | | | | 1.000 | NLT 0.99 |

Cont. .

**Table-8.9.4: Linearity of pantoprazole**

| *% level of standard* | *Detector response (area counts)* | | | | *Limit* |
|---|---|---|---|---|---|
| | *Inj. 1* | *Inj. 2* | *Inj. 3* | *Mean* | |
| 50 | 15899 | 15577 | 15539 | 15672 | |
| 75 | 24156 | 23803 | 23961 | 23973 | |
| 100 | 31839 | 32268 | 31300 | 31802 | |
| 125 | 39805 | 39939 | 39984 | 39909 | |
| 150 | 47862 | 47481 | 48092 | 47812 | |
| Slope | | | | 31933 | |
| Intercept | | | | -252.73 | |
| Coefficient of correlation (r) | | | | 1.000 | NLT 0.99 |

The results indicate that the detector response is linear. The results are shown graphically in Figure-151 to Figure-153

## 8.10 Method Precision

*Stock solution*

**Solution A** :25.07 mg of pantoprazole impurity A was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution B** :25.08 mg of pantoprazole impurity B was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution C** :12.54 mg of pantoprazole sodium WRS was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 mın, volume was made up with mobile phase.

**Solution D** :5 ml each of solution A and solution B was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

**Solution E** :5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase

**Method precision standard preparation:**

2 ml of solution D and solution E were transferred to a 50 ml volumetric flask, mixed and diluted up to mark with mobile phase. This solution was injected six times and mean and RSD of detector response of pantoprazole impurity A, pantoprazole impurity B and pantoprazole was calculated as shown in Table-8.10.1.

**Table-8.10.1: Detector response of method precision standard**

| *Injection* | *Area counts* | | | *Limit* |
|---|---|---|---|---|
| | *Impurity A* | *Impurity B* | *Pantoprazole* | |
| 1 | 62151 | 60768 | 33456 | NMT 5.0% |
| 2 | 63194 | 61462 | 35513 | |
| 3 | 64168 | 61166 | 35096 | |
| *4* | 63340 | 61174 | 33679 | |
| *5* | 64727 | 61374 | 33595 | |
| *6* | 65518 | 61397 | 35507 | |
| *Mean* | 63850 | 61224 | 34474 | |
| % RSD | 1.88 | 0.42 | 2.89 | |

Six sets of pantoprazole sodium sample were prepared on same day for analysis by the HPLC method as shown in Table-8.10.2.

**Table-8.10.2 : Dilution and concentration of pantoprazole sodium**

| *Sample No.* | *Wt. of WRS (mg)* | *Vol. of Stock solution D added (ml)* | *Final Dilution (ml)* | *Final concentration (µg/ml)* | | |
|---|---|---|---|---|---|---|
| | | | | *Impurity A* | *Impurity B* | *Pantoprazole* |
| 1 | 50.07 | 2 | 50 | 2.00 | 2.00 | 1001.4 |
| 2 | 50.21 | 2 | 50 | 2.00 | 2.00 | 1004.2 |
| 3 | 50 16 | 2 | 50 | 2.00 | 2.00 | 1003.2 |
| 4 | 50.14 | 2 | 50 | 2.00 | 2.00 | 1002.8 |
| 5 | 50.26 | 2 | 50 | 2.00 | 2.00 | 1005.2 |
| 6 | 50.31 | 2 | 50 | 2.00 | 2.00 | 1006.2 |

20 µl each of the above solutions was injected in duplicate into chromatographic system and mean area count was calculated. Results of these analyses are shown in Table-8.10.3.

**Table-8.10.3 : Results of method precision**

| *Set* | *Wt. of WRS (mg)* | *Final Dilution (ml)* | *Sample Concentration (µg/ml)* | *Observed Results in percentage* | | *Limit* |
|---|---|---|---|---|---|---|
| | | | | *Impurity A* | *Impurity B* | |
| M1 | 50.07 | 50 | 1001.4 | 0.211 | 0.191 | |
| M2 | 50.21 | 50 | 1004.2 | 0.195 | 0.192 | |
| M3 | 50.16 | 50 | 1003.2 | 0.203 | 0.191 | |
| M4 | 50.14 | 50 | 1002.8 | 0.194 | 0.197 | |
| M5 | 50.26 | 50 | 1005.2 | 0.209 | 0.192 | |
| M6 | 50.31 | 50 | 1006 2 | 0.194 | 0.193 | |
| Mean | | | | 0.201 | 0.193 | |
| RSD (%) | | | | 3.90 | 1.10 | NMT 5.0 % |

The relative standard deviation for impurity A is 3 9 % and for impurity B is 1.10 % obtained The method can therefore be said precise.

## 8.11 *Accuracy*

Recovery study was performed by spiking pantoprazole impurity A and pantoprazole impurity B in sample at 70, 85, 100, 115 and 130 % levels of 0.2 % of pantoprazole impurity A and pantoprazole impurity B.

***Stock solution***

**Solution A** :25.07 mg of pantoprazole impurity A was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution B** :25.08 mg of pantoprazole impurity B was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution C**:12.54 mg of pantoprazole sodium WRS was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution D** :5 ml each of solution A and solution B was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

**Solution E :**5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

Five sets of recovery samples were prepared for five level (70, 85, 100, 115 and 130% of target concentration) recovery study by transferring about 50 mg, accurately weighed, pantoprazole sodium standard into five 50 ml volumetric flasks separately. Appropriate volumes of stock solution was transferred into these flasks as shown in the Table–8.11.1 and diluted to volume with mobile phase.

**Table-8.11.1 : Recovery solution preparation**

| *% level of standard* | *Wt. of Pantoprazole sodium (mg)* | *Vol. of. Stock solution D (ml)* | *Final dilution (ml)* | *Final concentrations (µg/ml)* | |
|---|---|---|---|---|---|
| | | | | *Impurity A* | *Impurity B* |
| 70 | 50.17 | 1.4 | 50 | 1.404 | 1.404 |
| 85 | 50.22 | 1.7 | 50 | 1.705 | 1.705 |
| 100 | 50.05 | 2 0 | 50 | 2.006 | 2.006 |
| 115 | 50.11 | 2.3 | 50 | 2.306 | 2.307 |
| 130 | 50.08 | 2.6 | 50 | 2.608 | 2.608 |

20 µl of each of the above solutions was injected in triplicate into chromatographic system and mean area count was calculated. Results of these analysis are shown in Table–8.11.2

**Table-8.11.2 Recovery of pantoprazole impurity A from pantoprazole sodium**

| *% level of standard* | *Actual amount added (µg/ml)* | *Amount recovered (µg/ml)* | *% Recovery = Amt found ---------- x 100 Amt. Added* | *Limit* |
|---|---|---|---|---|
| 70 | 1.404 | 1.381 | 98.40 | 95.0 – 105.0% |
| 85 | 1.705 | 1.698 | 99.59 | |
| 100 | 2.006 | 2.076 | 103 52 | |
| 115 | 2.306 | 2.414 | 104.65 | |
| 130 | 2.608 | 2.708 | 103 87 | |
| *Mean* | | | 102.01 | |
| RSD (%) | | | 2.76 | NMT 5.0 % |

**Table-8.11.3 : Recovery of pantoprazole impurity B from pantoprazole sodium**

| *% level of standard* | *Actual amount added (µg/ml)* | *Amount recovered (µg/ml)* | *% Recovery = Amt found ---------- x 100 Amt. Added* | *Limit* |
|---|---|---|---|---|
| 70 | 1.404 | 1.406 | 100.10 | 95.0 – 105.0% |
| 85 | 1.705 | 1.716 | 100.62 | |
| 100 | 2.006 | 2.006 | 99.97 | |
| 115 | 2 307 | 2.412 | 104.52 | |
| 130 | 2.608 | 2.728 | 104 59 | |
| *Mean* | | | 101.96 | |
| RSD (%) | | | 2.33 | NMT 5.0 % |

## 8.12 *Limit Of Detection And Quantitation (LOD & LOQ)*

*LOD Stock solution*

**Solution A** :25.07 mg of pantoprazole impurity A was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution B** :25.08 mg of pantoprazole impurity B was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase After sonication for 10 min, volume was made up with mobile phase.

**Solution C** :12.54 mg of pantoprazole sodium WRS was weighed accurately and transferred to a 25 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution D** :5 ml each of solution A and solution B was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

**Solution E** :5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

Further dilutions were made as mentioned in Table-8.12.1

**Table-8.12.1 : Solution preparation for LOD and LOQ**

| *Level* | *Vol. of LOD stock soln (ml)* | *Final dilution (ml)* | *Final Concentrations (µg/ml)* | | |
|---|---|---|---|---|---|
| | | | *Pantoprazole impurity A* | *Pantoprazole impurity B* | *Pantoprazole* |
| D1 | 10 | 10 | 0 5014 | 0.5016 | 0.2508 |
| D2 | 5 | 10 | 0.2507 | 0 2508 | 0 1254 |
| D3 | 2 5 | 10 | 0 1254 | 0.1254 | 0 0627 |
| D4 | 1 2 | 10 | 0 0602 | 0.0602 | 0 0301 |
| D5 | 0.6 | 10 | 0 0300 | 0.0300 | 0 0151 |
| D6 | 0 3 | 10 | 0 0150 | 0.0075 | 0 0075 |
| D7 | 0.1 | 10 | 0.0050 | 0.0050 | 0.0025 |

20 µl of each of the above solutions was injected in triplicate into chromatographic system and mean area count and %RSD was calculated. Results of these analyses are shown in Table-8.12.2 .

**Table-8.12.2 : Limit of Detection and Limit of Quantitation Study (pantoprazole impurity A)**

| *Level* | *Conc. (µg/ml)* | *Inj. 1* | *Inj. 2* | *Inj. 3* | *Mean area* | *RSD (%)* |
|---|---|---|---|---|---|---|
| D1 | 0 5014 | 15275 | 15210 | 15395 | 15293 | 0 61 |
| D2 | 0.2507 | 7623 | 8310 | 8125 | 8019 | 4 43 |
| D3 | 0 1254 | 3612 | 4294 | 4478 | 4128 | 11 05 |
| D4 | 0 0602 | - | - | - | - | - |

**Table-8.12.3 : Limit of Detection and Limit of Quantitation Study (pantoprazole impurity B)**

| *Level* | *Conc. (µg/ml)* | *Inj. 1* | *Inj. 2* | *Inj. 3* | *Mean area* | *RSD (%)* |
|---|---|---|---|---|---|---|
| D1 | 0.5016 | 16886 | 16508 | 16208 | 16534 | 2 05 |
| D2 | 0.2508 | 7303 | 7786 | 7516 | 7535 | 3 21 |
| D3 | 0.1254 | 3357 | 3879 | 3910 | 3715 | 8.36 |
| D4 | 0.0602 | - | - | - | - | |

The limit of quantitation and detection for each component is as follows :

## 8.13 *Ruggedness*

Method ruggedness was established by analysing a sample spiked with pantoprazole impurity A and pantoprazole impurity B at target level (0.2 % i.e. about 2 µg/ml of pantoprazole impurity A and pantoprazole impurity B) in a previously analysed sample at normal operating condition and at changed conditions as mentioned below and results were compared.

Figure-160 to 165 shows typical chromatograms of ruggedness.

### 8.13.1 Under original condition

Sample was analysed under normal operating conditions as specified in method.

5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

| *Identification* | *Wt taken mg* | *ml* | | | *Conc.(µg/ml)* | | |
|---|---|---|---|---|---|---|---|
| | *Pantoprazole sodium* | *Vol. of stock soln. D* | *Vol. of stock soln. E* | *Final dilution* | *Pantoprazole Impurity A* | *Pantoprazole Impurity B* | *Pantoprazole sodium* |
| **Standard** | - | 2.0 | 2.0 | 50 | 2.01 | 2.01 | 1.00 |
| **Sample** | 50.31 | 2 | - | 50 | 2.01 | 2.01 | 1006.2 |

Figure-160 shows typical chromatograms of ruggedness.

### 8.13.2 Change in Analyst

Sample was analysed by changing analyst and keeping all other parameters same.

5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

| *Identification* | *Wt taken mg* | *ml* | | | *Conc.(µg/ml)* | | |
|---|---|---|---|---|---|---|---|
| | *Pantoprazole sodium* | *Vol. of stock soln. C* | *Vol. of stock soln.D* | *Final dilution* | *Pantoprazole Impurity A* | *Pantoprazole Impurity B* | *pantoprazole sodium* |
| **Standard** | - | 2 0 | 2.0 | 50 | 2.01 | 2.01 | 1.00 |
| **Sample** | 50.04 | 2 | - | 50 | 2.01 | 2.01 | 1000 8 |

Figure-161 shows typical chromatograms of ruggedness

### 8.13.3 Change in column

The sample was analysed by using RESTEK U S.A (C18, 250cm x 4 6mm, 5µ) and keeping all other parameter same.5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

| *Identification* | *Wt taken mg* | *ml* | | | *Conc (µg/ml)* | | |
|---|---|---|---|---|---|---|---|
| | *pantoprazole sodium* | *Vol of stock soln C* | *Vol of stock soln D* | *Final dilution* | *Pantoprazole Impurity A* | *Pantoprazole Impurity B* | *pantoprazole sodium* |
| **Standard** | - | 2.0 | 2 0 | 50 | 2.01 | 2.01 | 1.00 |
| **Sample** | 50.04 | 2 | - | 50 | 2.01 | 2.01 | 1000.8 |

Figure-163 shows typical chromatograms of ruggedness

### 8.13.4 Change in column temperature

The sample was analysed by changing the column temperature to 40°C instead of ambient temperature (25°C) and keeping all other parameter same

5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

| *Identification* | *Wt taken mg* | *ml* | | | *Conc.(µg/ml)* | | |
|---|---|---|---|---|---|---|---|
| | *Pantoprazole sodium* | *Vol. of stock soln. C* | *Vol. of stock soln. D* | *Final dilution* | *Pantoprazole Impurity A* | *Pantoprazole Impurity B* | *Pantoprazole sodium* |
| **Standard** | - | 2 0 | 2 0 | 50 | 2.01 | 2.01 | 1.00 |
| **Sample** | 50.12 | 2 | - | 50 | 2.01 | 2.01 | 1002 4 |

Figure-164 shows typical chromatograms of ruggedness

### 8.13.5 Change in mobile phase composition

The sample was analysed by changing mobile phase composition to 55 : 45 (buffer: methanol) instead of 50 . 50 (buffer : methanol ) and keeping all other parameters same

5 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

| *Identification* | *Wt taken mg* | *ml* | | | *Conc.(µg/ml)* | | |
|---|---|---|---|---|---|---|---|
| | *pantoprazole sodium* | *Vol. of stock soln. C* | *Vol. of stock soln.D* | *Final dilution* | *Pantoprazole Impurity A* | *Pantoprazole Impurity B* | *pantoprazole sodium* |
| **Standard** | - | 2.0 | 2.0 | 50 | 2.01 | 2.01 | 1.00 |
| **Sample** | 50.07 | 2 | - | 50 | 2.01 | 2.01 | 1001.4 |

Figure-162 shows typical chromatograms of ruggedness

### 8.13.6 Change in instrument

The sample was analysed by changing HPLC instrument (HPLC-21) and keeping all other parameters same. Following HPLC System was used for this study. LC-10AT pump, SIL –10ADvp autoinjector, SPD-10Avp detector, SCL-10Avp system controller and Class-vp series software and keeping all other parameters same.

4 ml of solution C was transferred in to a 100 ml volumetric flask. Mixed and diluted to volume with mobile phase.

Figure-165 shows typical chromatograms of ruggedness

| *Identification* | *Wt taken mg* | *ml* | | | *Conc.(µg/ml)* | | |
|---|---|---|---|---|---|---|---|
| | *pantoprazole sodium* | *Vol. of stock soln. C* | *Vol. of stock soln.D* | *Final dilution* | *Pantoprazole Impurity A* | *Pantoprazole Impurity B* | *Pantoprazole sodium* |
| **Standard** | - | 2.0 | 2.0 | 50 | 2.01 | 2.01 | 1.00 |
| **Sample** | 50.22 | 2 | - | 50 | 2.01 | 2.01 | 1004.4 |

**Result of Ruggedness of the method**

| *Parameter changed* | *Pantoprazole impurity A* | *Pantoprazole Impurity B* |
|---|---|---|
| Original condition | 0.214 | 0.203 |
| Change in analyst | 0.212 | 0.214 |
| Change in column | 0.210 | 0.213 |
| Change in column temperature | 0.214 | 0.211 |
| Change in mobile phase composition | 0.204 | 0.206 |
| Change in instrument | 0.204 | 0.224 |

## 8.14 Stability In Analytical Solution

**Solution A:**25.09 mg of pantoprazole impurity A and 25.14 mg of impurity B was weighed accurately and transferred to a 50 ml volumetric flask containing 10 ml of mobile phase. After sonication for 10 min, volume was made up with mobile phase.

**Solution B :**12.54 mg of pantoprazole sodium was weighed accurately and transferred to a 50 ml volumetric flask containing 10 ml of mobile phase After sonication for 10 min, volume was made up with mobile phase.

**Solution C :** 1 ml each of solution A and B was transferred in to a 10 ml volumetric flask. Mixed and diluted to volume with mobile phase.

20 µl of solution C was injected at different time intervals and peak areas were recorded.

**Stability of pantoprazole sodium in analytical solution**

| *Time (hrs)* | *Area counts* | *% deviation from mean initial area counts* | *Limit* |
|---|---|---|---|
| Initial | 776487 | - | |
| 09 | 777305 | 0.11 | |
| 32 | 776051 | 0.06 | |
| 56 | 773664 | 0.47 | |
| 70 | 769974 | 0.84 | NMT 5.0 % |

**Stability of pantoprazole impurity A in analytical solution**

| *Time (hrs)* | *Area counts* | *% deviation from mean initial area counts* | *Limit* |
|---|---|---|---|
| Initial | 1701515 | - | |
| 09 | 1716320 | 0.87 | |
| 32 | 1712440 | 0.64 | |
| 56 | 1621109 | 4.68 | |
| 70 | 1617001 | 4.97 | NMT 5.0 % |

**Stability of pantoprazole impurity B in analytical solution**

| *Time (hrs)* | *Area counts* | *% deviation from mean initial area counts* | *Limit* |
|---|---|---|---|
| Initial | 1462591 | 0 | |
| 09 | 1464634 | 0.14 | |
| 32 | 1460428 | 0.15 | |
| 56 | 1473504 | 0.61 | |
| 70 | 1468634 | 0.27 | NMT 5.0 % |

No significant change was observed in pantoprazole sodium and pantoprazole impurity A, pantoprazole impurity B peak areas upto 70 hrs. Hence the solutions need not be injected immediately.

### 8.15 Response Factor

Response factor for pantoprazole impurity A and pantoprazole impurity B was determined from the linearity plot of pantoprazole impurity A and pantoprazole impurity B.

| | *Pantoprazole sodium* | *Pantoprazole impurity A* | *Pantoprazole Impurity B* |
|---|---|---|---|
| **Slope** | 31933 | 43861 | 30481 |
| **Response factor *** | 1.0 | 1.37 | 0.95 |

*The response factor for pantoprazole impurity A and pantoprazole impurity B is calculated by using following formula.

$$F = \frac{S_{im}}{S_S}$$

F = Response factor for pantoprazole impurity A and pantoprazole impurity B

$S_{im}$ = Slope of pantoprazole impurity A and pantoprazole impurity B obtained from linearity study.

$S_S$ = Slope of pantoprazole sodium obtained from linearity study

### 8.16 Summary of validation

| **Acceptance limit** | | **Actual results** | | |
|---|---|---|---|---|
| **System suitability** | | | | |
| Resolution between pantoprazole impurity A and pantoprazole sodium | NLT 20.0 | | | |
| | | **Pantoprazole impurity A** | **Pantoprazole impurity B** | **Pantoprazole sodium** |
| **Precision** | | | | |
| Instrument precision - RSD (%) of Detector Response for pantoprazole impurity B and pantoprazole sodium | ≤ 5 0 % | 1 88 % | 0.42 % | 2 89 % |

| | | | | |
|---|---|---|---|---|
| **Method precision** - RSD (%) of pantoprazole impurity A | ≤ 5.0 % | 3.90 % | 1.10 % | - |
| **Linearity and Range** | | | | |
| Coefficient of correlation (r) | ≥ 0.99 | 1.000 | 1.000 | 1.000 |
| RSD (%) of detector responses | ≤ 5.0 % | 0.82 % max. | 0.34 % max. | 1.26% max. |
| **Accuracy** | | | | |
| Percentage recovery | 95.0 % - 105.0 % | 102.01 % | 101.96 % | - |
| **Minimum quantitation level (%)** | ≤ 5.0 % | 0.025 % | 0.025 % | 0.013 % |
| **Minimum detection level (%)** | | 0.013 | 0.013 | 0.006% |
| **Ruggedness** | *Difference NMT 10.0 % of impurity limit* | | | |
| Original condition | | 0.214 | 0.203 | |
| Change in analyst | | 0.212 | 0.214 | |
| Change in column | | 0.210 | 0.213 | |
| Change in column temperature | | 0.214 | 0.211 | |
| Change in mobile phase composition | | 0.204 | 0.206 | |
| Change in instrument | | 0.204 | 0.224 | |

The results of the study indicates that this method for related substances in pantoprazole sodium is precise, accurate, linear in detector response and rugged.

### 8.17 Assay method

**8.17.1 Method development:** Simple ammonium dihydrogen orthophosphate buffer and acetonitrile mixture is taken to optimize assay method. Finalised method is given below.

**Reagents :**

1) Ammonium dihydrogen orthophosphate : AR grade
2) Acetonitrile : HPLC grade
3) Orthophosphoric acid : AR grade
4) Water : Milli-Q grade

**Buffer solution** : Transfer 8.63 g of ammonium dihydrogen orthophosphate into a 1000ml volumetric flask, dissolve and dilute upto mark with milli-Q water. Adjust the pH of the solution to 3.0 (± 0.1) with orthophosphoric acid.

**Mobile phase :** Prepare filtered and degassed mixture of 750 volumes of buffer and 250 volumes of acetonitrile. Filter and degas prior to use.

**Standard preparation** :Transfer about 100 mg of accurately weighed, pantoprazole.WRS into a 50 ml volumetric flask, dissolve in and dilute upto mark with mobile phase. Transfer 5ml of this solution to 50ml volumetric flask, mix and dilute upto mark with mobile phase.

**Test preparation** :Transfer about 100 mg accurately weighed, sample into a 50 ml volumetric flask Dissolve in and dilute upto mark with mobile phase. Transfer 5 ml of this solution to 50ml volumetric flask, mix and dilute upto mark with mobile phase.

**Chromatographic condition :**

| | | |
|---|---|---|
| Column | | Hypersil C8,BDS (25 cm x 4.6mm) 5 µ, (Thermoquest, U K.) |
| Flow rate | . | 1.0 ml/min |
| Detector | · | UV set at 230 nm |
| Attenuation | : | Set appropriately |
| Run time | . | About 25 min. |
| Injection volume | . | 10 µl |

**Procedure**·

(1) Set up chromatographic system as described under instrumental conditions.

(2) Inject 10 µl of the standard solution six times and calculate the RSD of detector response The RSD of six injection should not be more than 2.0% and tailing factor of pantoprazole peak should not be more than 2.5.

(3) Inject 10 µl of test preparation in duplicate into the chromatograph and record the chromatograms.

(4) Retention time of pantoprazole is about 12 0 min

**Calculation :**

$$\text{\% Assay of pantoprazole. (on dried basis)} = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{DS} \times \frac{P \times 100}{100 - Q}$$

Where :

| | | |
|---|---|---|
| AT | = | Average area count of pantoprazole peak in test preparation |
| AS | = | Average area count of pantoprazole peak in standard preparation |
| WT | = | Weight of sample in mg |
| WS | = | Weight of pantoprazole standard in mg |
| DS | = | Dilution factor of standard preparation |
| DT | = | Dilution factor of test preparation |
| P | = | % purity of pantoprazole standard (as is basis) |
| Q | = | Loss on drying at 105 °C for 3 hrs. |

**Limit : 98.0 to 102.0 %**

**8.17.2 Validation Protocol**

**Scope** :This method upon validation, can be used for the analysis of assay in pantoprazole.

**Analytical method:** As per method given in section 8.17.1

**8.17.3 The experiments are designed to study**

a. Instrument precision
b Linearity and range
c Method precision
d Specificity (degradation study)
e Ruggedness of the method
f Solution stability

**8.17.4 Stock solutions :**

**Standard stock solution** :Transfer about 250 mg accurately weighed pantoprazole (working standard) into a 50 ml volumetric flask Dissolve in and dilute upto mark with mobile phase (5,000 µg/ml of pantoprazole)

**Standard solution:** Transfer about 100 mg accurately weighed pantoprazole (working standard) into a 50 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase. Pipette out 5 ml in to a 50ml volumetric flask, mix and dilute upto mark with mobile phase. (200µg/ml pantoprazole).

**Sample solution** :Transfer about 100 mg accurately weighed pantoprazole (working standard) into a 50 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase. Pipette out 5 ml into a 50ml volumetric flask, mix and dilute upto mark with mobile phase. (200µg/ml pantoprazole).

**Procedure**:

i) Set up the system as mentioned under the chromatographic conditions.
ii) Inject 10 µl of standard solution six times and sample solution in duplicate (As mentioned in section 2.3 & 2.4)
iii) Calculate the tailing factor of pantoprazole peak
iv) Calculate the relative standard deviation of the detector response for pantoprazole standard.

**Acceptance limit :**

The tailing factor of pantoprazole peak is NMT 2.5
RSD of detector response for pantoprazole standard is NMT 2.0%

**8.17.5 Method precision :**

i) Set up the system as mentioned under the chromatographic conditions. Inject 10 µl of the standard solution (as mentioned in section 2.3) six times and record the chromatograms upto 25 min.
ii) Prepare six sets of sample solution as directed under the method.
iii) Inject 10 µl of each sample solution in duplicate into the chromatography set to the condition mentioned under the method and record the chromatograms upto 25 min.
iv) Calculate the assay in each sample set.

**Acceptance criteria :**

RSD of the calculated assay in six sets should not be more than 2.0 %

**8.17.6 Linearity and range:**

**i)** Use standard stock solution (mentioned under section 8.17.4) for preparation of following solution

**ii) Linearity solutions.**

| *Sr. No.* | *%Assay level* | *Vol. of standard st. solution* | *final Dilution* | *Final concentrations of pantoprazole* |
|---|---|---|---|---|
| | | **ml** | **ml** | **µg/ml** |
| **L1** | 50 | 1.0 | 50 | 100 |
| **L2** | 75 | 1.5 | 50 | 150 |
| **L3** | 100 | 2.0 | 50 | 200 |
| **L4** | 125 | 2.5 | 50 | 250 |
| **L5** | 150 | 3.0 | 50 | 300 |

**iii)** Inject 10µl each of the linearity solution in triplicate into the chromatographic system set to the conditions mentioned under the method and record the chromatograms upto 25 min.

**iv)** Calculate the mean and RSD of the detector responses for each linearity level.

**v)** Plot a graph of the concentration versus mean area count and perform mathematical Regression for pantoprazole

**Acceptance limits:**

RSD of area count for pantoprazole at each level is less than 2.0 %.

Plot of concentration versus detector response for each component is linear.

The linear regression ($r^2$) for pantoprazole is more than 0.999.

**8.17.7 Specificity of the method** :Specificity of the method established by demonstrating no interference from degradation products. This is demonstrate by carrying out forced degradation of the sample by adding 5.0M HCl, 5.0M NaOH, 30 % $H_2O_2$, 1 %$KMNO_4$ separately The sample is also subjected to exposure under UV light for 48 hrs and SUN-light for 8 hrs and oven degradation by heating it in a oven at specific temperature for 4 hrs. Prepare samples as given below and inject into HPLC with a Shimadzu SPD M-10A photodiode array detector. Record the chromatograms upto 25 min. to check for degraded peaks. In each case peak purity of pantoprazole is determine to examine interference from degradation products.

**8.17.7.1 Acid degradation** :Transfer 20 mg pantoprazole into a 100 ml volumetric flask containing 5 ml water Swirled to disperse. To this add 5 ml of 5.0M HCl. Keep the sample for 48 hrs. After that, adjust the pH of solution to 7.0 with 5.0M NaOH and make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole peak by diode array detector.

**8.17.7.2 Alkali degradation** : Transfer 20 mg pantoprazole into a 100 ml volumetric flask containing 5 ml water. Swirl to disperse. To this add 5 ml of 5.0M NaOH. Keep the sample for 48 hrs. After that, adjust the pH of solution to 7.0 with 5.0M HCl and make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole peak by diode array detector.

**8.17.7.3 Peroxide degradation** :Transfer 20 mg pantoprazole into a 100 ml volumetric flask containing 5 ml water. Swirl to disperse. To this add 1 ml of 30 % $H_2O_2$. Keep the sample for 48 hrs. After that, make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole peak by diode array detector.

**8.17.7.4 Degradation with $KMnO_4$** : Transfer 20 mg pantoprazole into a 100 ml volumetric flask containing 5 ml water. Swirl to disperse. To this add 1 ml of 1.0% $KMnO_4$. Keep the sample for 48 hrs. After that, make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole by diode array detector

**8.17.7.5 Degradation under UV light** :Transfer 20mg pantoprazole (previously kept under UV light at 254 nm for 48 hrs.) into a 100 ml volumetric flask containing 5 ml water. Swirl to disperse. After that, make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole peak by diode array detector

**8.17.7.6 In oven at 105°C for 3 hrs. degradation** : Transfer 20 mg pantoprazole (previously kept under Oven at 105°C for 3 Hrs.) into a 100ml volumetric flask containing 5 ml water. Swirled to disperse. After that, make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole peak by diode array detector.

**8.17.7.7 Degradation under SUN light :** Transfer 20mg pantoprazole (previously kept under SUN light for 8 hrs.) into a 100 ml volumetric flask containing 5 ml water. Swirl to disperse. After that, make up the volume with mobile phase. Inject this solution into the HPLC. Determine the peak purity of pantoprazole peak by diode array detector.

**Acceptance limits :** Peak purity of pantoprazole peak should be grater than 99.0%

**8.17.8 Stability in analytical solution :**

Inject the Solution of standard preparation (200µg/ml) at different time intervals and record the peak areas.

**Acceptance limit :**

RSD of pantoprazole peaks at different time intervals should not be more than 2 0%

**8.17.9 Ruggedness :**

The assay of a sample carried out by deliberately changing some of the parameters should not differ by more than ± 0.5 %.

**8.18 Validation-Experimental**

**Reagents and chemicals :**

1) Ammonium dihydrogen orthophosphate : AR grade (Ranbaxy chem Ltd.)
2) Orthophosphoric acid : AR grade (Ranbaxy chem Ltd.)
3) Acetonitrile . HPLC grade (Ranbaxy chem Ltd.)
4) Water : Milli-Q grade

**Working standard and sample :**

1) Pantoprazole WRS : B No Vz-0030600 (Hetero drugs)
2) Pantoprazole sample B No Vz-020999 (Hetero drugs)

**Chromatographic system :**

Column : 4 6mm x 25cm, 5µm, Hypersil C8 (Shandon, U K)

| | | |
|---|---|---|
| Detector | : | UV-230 nm |
| Flow rate | : | 1.0 ml |
| Injection volume | : | 10 µl |
| Run time | : | 25 min. |

- Shimadzu LC-10AT$_{VP}$ solvent delivery pump with SIL-10A$_{VP}$ Autoinjector.
- Shimadzu SPD-10A$_{VP}$ UV detector.
- *VP*-SERIES computer software .

**Buffer solution** :43.15 g. ammonium dihydrogen orthophosphate was transferred into a 5.0 lit beaker. To this 5000 ml Milli-Q water was added by a measuring cylinder and dissolved, pH was adjusted to 3.02 with orthophosphoric acid.

**Mobile phase :** 6 Lit of mobile phase prepared by mixing 4500 ml of buffer solution, and 1500 ml of acetonitrile. Entire mixture was filtered and degassed.

**Stock solution :**

Standard stock solution :250.91 mg pantoprazole (WRS) transferred into a 50ml volumetric flask, dissolved in and diluted to volume with mobile phase (5001.2 µg/ml of pantoprazole standard).

## 8.19 Results and Discussions :

**8.19.1 Instrument precision** :The Instrument precision was checked by injecting the pantoprazole standard preparation 200.02µg/ml (100 01mg was dissolved in 50 ml mobile phase, transferred 5ml of this solution to 50ml volumetric flask and diluted to volume with mobile phase.) six times. Individual area counts and % RSD values are shown in Table - 8.19.1.1 below : [Limit· RSD NMT 2.0 %]

Figure-166 shows typical chromatograms of system precision

**Table – 8.19.1.1 : Results of Instrument precision study**

| Injection | Pantoprazole Area counts |
|---|---|
| 1 | 3318897 |
| 2 | 3323188 |
| 3 | 3331901 |
| 4 | 3214972 |
| 5 | 3379227 |

| | |
|---|---|
| 6 | 3306315 |
| Mean | 3312417 |
| Standard deviation | 53915 |
| Relative standard deviation | 1.63 |

**8.19.2 Linearity study** :The linearity of detector (UV) response for pantoprazole was determined by preparing and injecting solutions in the concentration range of 100 –300 µg/ml (50-150 % of assay conc.) for pantoprazole standard. Figure-109 shows linearity graphs of pantoprazole, Figure-170 shows linearity chromatogram set-1 and Table-8.19.1.1 shows values of slope, intercept and regression of linear plot.

**Table - 8.19.1.1 : Linearity study**

| *Sr. No.* | *Vol. of Standard stock solution* | *Final dilution* | *Final conc.* | *Area counts* | | | *Mean Area counts (n = 3)* | *RSD (%)* |
|---|---|---|---|---|---|---|---|---|
| | *(ml)* | *(ml)* | *(µg/ml)* | *1* | *2* | *3* | | |
| L1 | 1.0 | 50 | 100 36 | 1785714 | 1824958 | 1844363 | 1818345 | 1.64 |
| L2 | 1.5 | 50 | 150.54 | 2699056 | 2754187 | 2449380 | 2734208 | 1.12 |
| L3 | 2.0 | 50 | 200.72 | 3668095 | 3672482 | 3685570 | 3675382 | 0.25 |
| L4 | 2 5 | 50 | 250 90 | 4580375 | 4577587 | 4569246 | 4575736 | 0.13 |
| L5 | 3 0 | 50 | 301.08 | 5482973 | 5476117 | 5447638 | 5468909 | 0.34 |
| Slope | | | | | | | 18219 | |
| Intercept | | | | | | | -2547 | |
| Linear regression ($r^2$) | | | | | | | 0 9999 | |

[Limit: Linear regression ($r^2$) - NLT 0.999]

[Limit: RSD of triplicate injections - NMT 2.0 %]

**8.19.3Method precision** :Six sets of pantoprazole sample (B.No.1315/113/49) were prepared on same day for analysis by the HPLC method. Results of these analysis are shown in Table –8.19.2.1

**.Table - 8.19.2.1 : Results of method precision**

| | *Final conc. (µg/ml)* | *Area of individual injection* | | | *% Assay of pantoprazole (As is basis)* |
|---|---|---|---|---|---|
| | | *1* | *2* | *Mean Area counts* | |
| Standard B.No. | | i)3375533 | ii)3394049 | 3420374 | |
| | | iii)3422758 | iv)3400048 | | |

| (Vz-0030600) | | v)3461971 | vi)3467885 | | |
|---|---|---|---|---|---|
| Sample B.No. (Vz-020999) | | | | | |
| 1 | | 3420991 | 3413452 | 3420991 | 99.52% |
| 2 | | 3414605 | 3406774 | 3410690 | 99.39% |
| 3 | | 3472854 | 3444731 | 3458793 | 99.58% |
| 4 | | 3532178 | 3547900 | 3540039 | 99.76% |
| 5 | | 3460594 | 3511590 | 3486092 | 99.74% |
| 6 | | 3662068 | 3678430 | 3670249 | 100.19% |
| **Mean** | | | | | 99.70% |
| Standard deviation | | | | | ± 0.25 |
| Relative standard deviation | | | | | 99.70 % |

[Limit : RSD of six injections of standard - NMT 2.0 %]
[Limit : % recovery of each levels 98.0 % - 102.0 %]
[Limit : RSD for recovery levels - NMT 2.0 %]

Figure-168 shows typical chromatograms of method precision set-1

**8.19.4 Specificity of the method** :Specificity of the method was established by demonstrating no interference from degradation products. This was demonstrated by carrying out forced degradation of the sample by adding 10M HCl, 10M NaOH, 30 % $H_2O_2$, 1 % (w/v) $KMnO_4$ separately. The sample was also subjected to exposure under UV light for 48 hrs, sunlight exposure for 8 hrs. and oven (105°C) degradation by heating it in a oven at 105°C for 4 hrs. The samples were prepared as given below and were injected into HPLC with a Shimadzu SPD M-10$A_{VP}$ photodiode array detector. The chromatograms were recorded upto 25 min. to check for degraded peaks. In each case peak purity index of pantoprazole was determined to examine interference from degradation products. .

**8.19.4.1 Acid degradation** : 20.15mg pantoprazole was transferred into a 100ml volumetric flask containing 5 ml water Swirled to disperse. To this 5 ml of 10M HCl was added. The sample was kept for 48 hrs. After that, pH of the solution was adjusted to 7.0 with 10M NaOH and volume was made up with mobile phase. This solution was injected into the HPLC. Figure-118 shows HPLC chromatograms for acid degradation. Peak purity of SUN-1315peak as determined by diode array detector was > 99.0% Significant degradation was found as determined by comparing area counts with the

standard preparation. However, the degradation do not interfere with pantoprazole peak as shown by peak purity .

**8.19.4.2 Alkali degradation** : 20.99 mg pantoprazole was transferred into a 100 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 5 ml of 10M NaOH was added. The sample was kept for 48 hrs. After that, pH of the solution was adjusted to 7.0 with 10 M HCl and volume was made up with mobile phase. This solution was injected into the HPLC. Figure-174 shows HPLC chromatogram for alkali degradation. Peak purity of pantoprazole peak as determined by diode array detector was > 99.0%. No Significant degradation was observed as compared with standard area..

**8.19.4.3 Peroxide degradation** :20.53 mg pantoprazole was transferred into a 100 ml volumetric flask containing 5 ml water Swirled to disperse. To this was added 1 ml of 30 % $H_2O_2$ was added. The sample was kept for 48 hrs. After that, volume was made with mobile phase. This solution was injected into the HPLC. Figure-175 shows HPLC chromatogram for peroxide degradation. No Significant degradation was found as determined by comparing area counts with the standard preparation. Peak purity of pantoprazole peak as determined by diode array detector was 99.96% .

**8.19.4.4Degradation with $KMnO_4$ :**

19.93 mg SUN-1315was transferred to 100 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 1ml 0.1M $KMnO_4$ was added. The sample was kept for 48 hrs. After that the volumes were made with mobile phase and filtered. This solution were injected into the HPLC. Peak purity of pantoprazole as determined by diode array detector was > 99.0% Significant degradation was observed as compared with standard area. However, the degradation do not interfere with pantoprazole peak as shown by peak purity index.

**8.19.4.5 Degradation under UV light :**

20.69 mg pantoprazole (previously kept under UV light at 254 nm for 48 hrs.) was transferred into a 100 ml volumetric flask containing 5 ml water, dissolved in and diluted to volume with mobile phase. This solution was injected into the HPLC. Figure-176 shows HPLC chromatogram for degradation under UV light. Peak purity of pantoprazole

peak as determined by diode array detector was > 99.0% .However no significant degradation was observed.

**8.19.4.6 In oven at 105 °C for 3 hr. degradation :**

20.98 mg pantoprazole was transferred into a 50ml beaker. The sample was heated in oven at 105°C for 3 hr. After cooling sample was transferred into 100 ml volumetric flask and volume was made with mobile phase. This solution was injected into the HPLC. Figure-178 shows HPLC chromatogram for oven degradation. Peak purity of pantoprazole as determined by diode array detector was > 99.0%. No significant degradation was observed.

**8.19.4.7 Degradation under Sun light (8Hrs) :**

20.96 mg pantoprazole (previously kept under Sun light for 8 hrs.) was transferred into a 50 ml volumetric flask containing 5 ml water, dissolved in and diluted to volume with mobile phase. This solution was injected into the HPLC. Figure-177 shows HPLC chromatogram for degradation under UV light. Peak purity of pantoprazole peak as determined by diode array detector was > 99.0% .However no significant degradation was observed.

**Table – 8.19.4 : Specificity of the method**

| *Degradation* | *Condition* | *% Residual drug.* | *Peak purity* | *Acceptance criteria (Peak purity)* |
|---|---|---|---|---|
| Acid degradation | 20.15 mg pantoprazole and 5 ml 1.0M HCl kept for 48 hrs. | 50.65% | 100.00 % | NLT 99.0% |
| Alkali degradation | 20.99 mg pantoprazole and 5 ml 1.0M NaOH kept for 48 hrs. | 100.76% | 100.00% | NLT 99.0% |
| Peroxide degradation | 20.24mg pantoprazole and 5ml of 30% $H_2O_2$ kept for 48 hrs. | 92.27% | 100.00% | NLT 99.0% |
| UV Light exposure | 19.59mg pantoprazole kept for 48 hrs. in UV exposure | 99.67% | 100.00% | NLT 99.0% |
| Thermal degradation | 20.92mg pantoprazole kept for 6 hrs. at 105°C in oven | 99.34% | 100.0% | NLT 99.0% |
| SUN-Light exposure | 19.68 mg pantoprazole kept for 8Hrs. in SUN-light | 98 84% | 99.94 % | NLT 99.0% |

### 8.19.5 Ruggedness :

Method ruggedness was determined by analysing same sample (# VZ-020999) at normal operating conditions and also by changing some operating analytical conditions such as instrument and analyst. Figure-173,169 shows HPLC chromatogram for sample at two different conditions.

| **Parameter** : | **Normal condition** | **Changed condition** |
|---|---|---|
| Column | Hypersil $C_8$, 25cm x 4.6mm,5 µ, (Shandon, U.K.) | Lichrospher RP-8e 25cmx 4.6mm,5 µ, (Merck, Germany.) |
| Flow rate : | 1.0 ml/min. | 1.2 ml / min. |
| Mobile phase : | Buffer : 750<br>Acetonitrile : 150 | Buffer : 745<br>Acetonitrile : 155 |
| Pump : | LC-10AT | LC-10AS |
| Detector : | SPD-10Avp | SPD - 10A |
| Software : | VP-series Software HPLC-12 | Class-LC-10 HPLC-10 |
| Injection volume : | 10µl | 10µl |
| Analyst . | NRP | **A.L.Prasad** |
| *Retention time:* | 11.96min. | 9 95min. |
| *Assay* : | 99.69 % | 99.36 % |

**Conclusion** :No significant change in assay was found even under the deliberately change conditions. Thus the ruggedness of the method is established.

### 8.19.6 Stability in analytical solution :

Solution of standard (200.34 µg/ml) was injected at different time intervals and peak areas were recorded.

**Table – 8.19.6.1 : Stability of drug in analytical solution**

| Time (hrs.) | pantoprazole (peak area) |
|---|---|
| INITIAL | 3491971 |
| 6 Hrs. | 3414228 |
| 14Hrs. | 3456256 |
| Mean | 3454152 |
| Relative standard deviation (%) | 1.13 % |

No significant change was observed for pantoprazole peak areas upto 14 hrs.

Figure-167 shows typical chromatograms of solution stability study

**8.19.7 Summary and conclusions :**

**Table - 8.19.7.1 : Validation results**

| *Sr. No.* | *Acceptance criteria* | *Observed value* | *Limit* |
|---|---|---|---|
| 1. | System suitability Tailing factor of pantoprazole peak | 1 87 | Tailing factor NMT.2.5 |
| 3 | Linearity range | Linear regression ($r^2$) = 0.9999 | Linear regression ($r^2$) NLT 0.999 |
| 4. | Instrument Precision | RSD : 1.63 % | RSD · NMT 2.0 % |
| 5. | Method precision | Assay :99.48%<br>RSD : 0 125 % | RSD .NMT 2..0 % |
| 6 | Ruggedness | Variation = 0 11 % | Variation ± 0.5 % |

All these observations indicate that this method for assay of pantoprazole is specific, accurate, precise and also stability indicating

**Recommendation And Limitation:** This method can be used for analysing pantoprazole assay .