CHAPTER-IV

STUDY THE IMPURITY PROFILE AND POLYMORPHISM OF OMEPRAZOLE

4.1 Introduction

Omeprazole 1, is a proton pump inhibitor (PPIs) and inhibits the action of hydrogen/potassium adenosine triphosphatase (H+/K+ATPase) in parietal cells.$^{1-5}$ It reduce the stomach acid by blocking the enzyme system responsible
for active transport of acid into the gastrointestinal lumen, H(+)/K(+) ATPase of the gastric parietal cell, also known as the “proton pump”.6

Omeprazole was the first drug in this class introduced in 1988. Since then, four other PPIs viz lansoprazole, rabeprazole, pantoprazole and esomeprazole have been introduced in 1995, 1999, 2000 and 2001 respectively. PPIs are used to treat peptic ulcers (duodenal and gastric), gastroesophageal reflux disease (GERD) and drug-induced ulcers (e.g., non-steroidal anti-inflammatory drugs {NSAIDs}). For peptic ulcer disease, PPIs are given with antibiotics to eradicate *Helicobacter pylori* (*H. pylori*), the bacteria that cause ulcers. For gastroesophageal reflux, which causes heartburn and acid regurgitation, the American gastroenterology association recommends that patients first try lifestyle modifications and over-the-counter medicines. Lifestyle modifications include avoiding foods, beverages and medicines that can aggravate heartburn, decreasing the size of portions at mealtimes, avoiding tight-fitting, clothing, losing weight if overweight and eating at least 3 hours before going to sleep. Over-the-counter medications include antacids and histamine-2-receptor antagonists (H2-RAs, commonly called “H2-blockers”), such as cimetidine or ranitidine. If these lifestyle changes and over-the-counter medications do not completely control heartburn symptoms, PPIs or high doses of H2-RAs may be prescribed.

Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GERD) and Zollinger-Ellison syndrome. It was first marketed by AstraZeneca as the magnesium salt of omeprazole under the trade names Losec and Prilosec and is now also available from generic manufacturers under various trade names. It is
one of the widely prescribed drugs internationally and it is available over the counter in some countries. Omeprazole is a substituted benzimidazole, 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole, a compound that inhibits gastric acid secretion.

Chemical Structure

Omeprazole 3D structure

All the prazoles of antiulcerative drugs especially free base are very sensitive to the heat and acidic conditions. A problem with omeprazole freebase is its stability characteristics. Upon storage without any precautions being taken, it is reported to be degraded at an undesirably high rate (during storage under accelerated stability conditions, e.g. at 37 °C). As the omeprazole is very sensitive, it is very important to known the clear picture of the impurities.

Due to the huge requirement of omeprazole in the market, it is very important to synthesize and show the level of the impurities in omeprazole to the customers. This makes us to give a complete study of omeprazole impurities in the present work.

4.2 Present work (Impurity Profile of Omeprazole)

In general the preparation of omeprazole sodium 5 involves three steps. In the first step the omeprazole sulphide 4, (2-[[4-(methoxy)-3,5-dimethyl-2-pyridinyl]methyl]thio]-1H-benzimidazole) is prepared by condensation of 5-
methoxy-1H-benzimidazole 2 and 2-chloromethyl-4-(methoxy)-3,5-dimethyl pyridine 3 by using sodium hydroxide in presence of water and acetone. In the second step oxidation of 2-[[4-(methoxy)-3,5-dimethyl-2-pyridinyl]methyl]thio]-1H-benzimidazole 4 with m-chloroperbenzoic acid or hydrogen peroxide to afford 1, which is further converted to its sodium salt of 1 by using sodium hydroxide in the presence of methanol (Scheme: 4.1).

Scheme: 4.1

While conducting experiments in the laboratory for the preparation of omeprazole sodium 5 we have found six impurities in isocratic high performance liquid chromatographic (HPLC) method whose area percentage
ranged from 0.08 to 0.35%. The same samples were analyzed by LC-MS method and identified peaks of Impurity 4 m/z 329, Impurity 6 m/z 361, Impurity 7 m/z 361, Impurity 8 m/z 495, Impurity 9 m/z 377, Impurity 10 m/z 314. These impurities were synthesized individually and characterized based on the spectroscopic data (HPLC, IR, NMR and MS). The structures of these impurities were shown in (Fig-4.1).

![Chemical structures of impurities](image)

**Figure 4.1** Impurities of Omeprazole

### 4.2.1 The synthetic pathways of impurities formation

The intermediate used in the preparation of omeprazole 1 is omeprazole sulphide 4, the some amount of the starting material 4 remains as unreacted with oxidizing reagent and carry forward for the next stages which result in the occurrence of impurity 4 at the final stage. During the oxidation of 1, due to
the over-oxidation, result in the formation of impurity 6, 7 and 9. In the preparation of the intermediate omeprazole sulphide 4, the condensation reaction occurs between the chloro compound 3 (2-chloromethyl-3,5-dimethyl-4-(methoxy)pyridine hydrochloride) and compound 4 in the presence of basic medium to form 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl]methane]thio]-1-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-1H-benimidazole 11 which undergoes oxidation to form impurity 8. In the preparation of key starting material 2-chloromethyl-4-(methoxy)-3,5-dimethyl pyridine 3, the starting material used is 2,3,5-collidine-1-oxide (3c). The starting material remains as unreacted while doing nitration and which reacts with compound 2 in further stage to form as 2-[[RS]-[(3,5-dimethyl-2-pyridinyl)methyl]thio]-5-methoxy-1H-benimidazole 12 which undergoes oxidation to form impurity 10.
4.2.2 Synthesis of impurities

The impurity 4 is prepared by the condensation of 2 with 3 in the presence of aqueous sodium hydroxide at room temperature. IR spectrum displayed characteristic absorptions at 1635, 1594 cm\(^{-1}\) corresponding to C=C, C=N stretching. The peaks at 1263 and 1079 cm\(^{-1}\) are indicative of ether functionality. In \(^1\)H NMR spectrum the S-alkylated methylene group shows the signal at \(\delta\) 4.65 as a singlet. DEPT spectra displayed one negative signal due to
one methylene group. In the mass spectrum showed peaks at $m/z$ 329, corresponding to molecular ion ($M^+$). Based on the spectral data the structure was characterized as 5-methoxy-2-[[4-methoxy-3,5-dimethylpyridin-2-yl]methyl]thio]-1H-benzimidazole 4.

**Scheme: 4.2**

The impurity 6 is prepared by the oxidation of omeprazole 1 with an oxidizing reagent $m$-CPBA in chloroform at ambient temperature for 1 h. IR spectrum displayed characteristic absorptions 3450 cm$^{-1}$ corresponding to the N-H group of imidazole, 1330 cm$^{-1}$ corresponding to O=S=O stretching. In $^1$H NMR spectrum the S-alkylated methylene group shows the signal at $\delta$ 5.04 as a singlet. In the mass spectrum displayed molecular ion at $m/z$ 361, which is 16 amu more than that of omeprazole. Based on the spectral data the structure is characterized as 5-methoxy-2-[[4-methoxy-3,5-dimethylpyridin-2-yl]methyl]sulphonyl]-1H-benzimidazole (6).

**Scheme: 4.3**

The impurity 7 is prepared by oxidation of 3 with $m$-CPBA, to give 7a followed by condensation with 2 in the presence of aqueous sodium hydroxide to give 7b which on further oxidation with $m$-CPBA in chloroform give impurity
7. In the IR spectrum shows characteristic peaks at 3440 cm\(^{-1}\) corresponding to the N-H group of imidazole, 1267 cm\(^{-1}\) corresponding to N-O and 1064 cm\(^{-1}\) corresponding to S=O. In \(^1\)H NMR spectrum the aromatic proton beside the nitrogen of pyridine show the signal at \(\delta\) 7.55 as singlet, S-alkylated methylene group show the signal at \(\delta\) 4.82 as singlet. In the mass spectrum displayed molecular ion at \(m/z\) 361, which is 16 amu more than that of omeprazole. Based on the spectral data the compound characterized as 5-methoxy-2-[[RS]-(4-methoxy-3,5-dimethyl-2-pyridinyl-1-oxide)methyl]sulfinyl]-1H-benzimidazole (Impurity 7, N-oxide).

Scheme: 4.4
Condensation of 4 with 3 to give compound 11, which on further oxidation furnished impurity 8. Impurity 8 displayed IR spectrum characteristic absorptions 1154 cm\(^{-1}\) is assignable to S=O stretching. The spectral data of impurity 8 have several additional resonances both in the aliphatic and aromatic region. The molecular ion at \(m/z\) 494 can be attributed to the \(N\)-aralkylated product of omeprazole 1 and 2-chloromethyl-3,5-dimethyl-4-(methoxy)pyridine moieties 3. In \(^1\)H NMR spectrum the protons at the adjacent of nitrogen in the two pyridine moieties show the signals at \(\delta\) 8.21 and \(\delta\) 8.19 as two singlets. The two methoxy groups show two signals at \(\delta\) 3.64/s and the four methyl groups show two signals at \(\delta\) 2.25/s and another two signals at \(\delta\) 2.15/s. Based on the above spectral data the molecular formula of impurity 8 could be C\(_{26}\)H\(_{30}\)N\(_4\)O\(_4\)S and the corresponding structure was characterized as 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl)methane]sulfinyl]-1-[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-1\(H\)-benzimidazole.

Scheme: 4.5
On comparison of impurity 6 with impurity 9, impurity 9 displayed molecular ion at \( m/z \) 377, which is 16 amu more than that of sulphone impurity 6 (\( m/z \) 361). Impurity 9 is prepared by the oxidation of impurity 7 with \( m \)-CPBA. Impurity 9 displayed IR spectrum characteristic absorptions at 3391 cm\(^{-1}\) corresponding to N-H group of imidazole, 1268, 1309 cm\(^{-1}\) corresponding to N-O, O=S=O stretching. In \(^1\)H NMR spectrum the proton at the adjacent of nitrogen in the pyridine give the signal at \( \delta \) 8.05/s. Based on the above spectral data the molecular formula of impurity 9 could be C\(_{17}\)H\(_{19}\)N\(_3\)O\(_5\)S and the corresponding structure was characterized as 5-methoxy-2-\{[(4-methoxy-3,5-dimethyl-2-pyridinyl-1-oxide)methyl]sulphonyl\}-1\(H\)-benzimidazol. The spectral data of omeprazole 1 is compared with those of impurities 4 to 10. It is interesting to note that impurities 4, 6, 7 and 9 have same skeletal system as evident by the number of NMR signals.

**Scheme: 4.6**
The impurity 10 is prepared by the condensation of compound 2 with 12 to give compound 13, which on further oxidation with m-CPBA to give impurity 10. Impurity 10 displayed IR spectrum characteristic absorptions at 3433 cm\(^{-1}\) corresponding to N-H stretching. It is also interesting to note that impurity 10 displayed molecular ion at \(m/z\) 315 with 30 amu less than that of omeprazole. In \(^1\)H NMR spectrum the presence of five aromatic proton in the region \(\delta\) 6.92-8.19 and the absence of methoxy group give the evidence as the impurity could be des methoxy of omeprazole. Based on the above spectral data the molecular formula of impurity 10 is C\(_{17}\)H\(_{19}\)N\(_3\)O\(_2\)S and the corresponding structure was characterized as 5-methoxy-2-[[\((R)\)-[3,5-dimethyl-2-pyridinyl] methyl]sulfinyl]-1\(H\)-benzimidazole.

**Scheme: 4.7**
The impurities were synthesized, subsequently characterized and were co-injected with the sample containing impurities and are found to be matching with the impurities in the sample. Based on their spectral data (IR, MS and NMR), these impurities were characterized as 5-methoxy-2-[((4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]-1H-benzimidazole (Impurity 4, ufiprazole); 5-methoxy-2-[((4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphonyl]-1H-benzimidazole (Impurity 6, sulphone); 5-methoxy-2-[[((RS)-(4-methoxy-3,5-dimethyl-2-pyridinyl-1-oxide)methyl]sulfinyl]-1H-benzimidazole (Impurity 7, N-oxide); 5-methoxy-2-[((4-methoxy-3,5-dimethyl-2-pyridinyl)methane]sulfinyl]-1-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-1H-benzimidazole (Impurity 8, N-aralkylated); 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl-1-oxide)methyl]sulphonyl]-1H-benzimidazole (Impurity 9, N-oxide sulphone); 5-methoxy-2-[[((RS)-(3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (Impurity 10, desmethoxy).

4.2.3 Spike of impurities 4, 6, 7, 8, 9, 10 with omeprazole sodium

A typical analytical LC chromatogram of a laboratory batch of omeprazole sodium bulk drug was recorded using the LC method. The target
impurities under study are marked as impurity 4 retention time (RT): 21.34, molecular weight (MW): 329), impurity 6 (RT: 7.58, MW: 361), impurity 7 (RT: 4.01, MW: 361), impurity 8 (RT: 33.30, MW: 494), impurity 9 (RT: 3.16, MW: 377), impurity 10 (RT: 8.52, MW: 315). The LC-MS compatible method is described in which all the impurities are used to detect Fig: 4.3. The structures of these impurities are shown in Fig: 4.1. Impurities 6, 7, 9 and 10 are polar and impurities 4 and 8 are non-polar with respect to omeprazole sodium.

Fig: 4.3 HPLC chromatogram of omeprazole sodium 5 laboratory sample spiked with six impurities.

4.3 Experimental Section

The IR spectra were recorded in the solid state as KBr dispersion medium using Perkin-Elmer Spectrum One FT-IR spectrophotometer. The LC-MS has been performed on AB-4000 Q-trap LC-MS/MS mass spectrometer (MDS SCIEX).\(^\text{18}\) \(^1\)H NMR was recorded in DMSO-\(d_6\) using 200 MHz and 400
MHz Varian Mercury plus 400 MHz FT NMR spectrometer in DMSO-\textit{d}_6. The $^1$H chemical shift values were reported on the $\delta$ scale in ppm, relative to TMS ($\delta = 0.00$ ppm).

**Synthesis of Impurity 4**

5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]-1H-benzimidazole 4 is the precursor of omeprazole which was prepared by condensation of 5-methoxy-1H-benzimidazole 2 (50 g, 0.27 mol) with 2-chloromethyl-4-(methoxy)-3,5-dimethyl pyridine 3 (51.8 g, 0.27 mol) by using sodium hydroxide (27.7 g, 0.69 mol) in presence of water and acetone at ambient temperature. The separated solid was filtered and dried to give 82 g of compound 4.

**Yield:** 91%.

**FT-IR (cm$^{-1}$):** 3433 (N-H), 3073 (Ar-H), 2959 (C-H), 1635, 1594 (C=C, C=N), 1569 (N-H) 1456 (C-H), 1079 (C-O), 836 (C-H).

**$^1$H NMR $\delta$ ppm:** 2.20 (s, 3H, CH$_3$), 2.27 (s, 3H, CH$_3$), 3.73 (s, 3H, OCH$_3$), 3.77 (s, 3H, OCH$_3$), 4.65 (s, 2H, CH$_2$), 6.76 (dd, 1H, Ar-H), 6.98 (s, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 12.25 (s, N-H).

**Mass: (m/e) 330(M$^+$+H).**

**Synthesis of Impurity 6**

To a solution of omeprazole 1 (50 g, 0.144 mol) in chloroform (250 ml), $m$-CPBA (25 g, 0.144 mol) dissolved in 100 ml of chloroform was added at ambient temperature for 1 h then the reaction mass was stirred for 45 min. After completion of the reaction (vide TLC) the reaction mixture was quenched
with a solution of sodium hydroxide (15 g) and water (300 ml). The chloroform was separated and discarded, the resulting mixture was cooled to 0-5 °C and pH of the solution was adjusted to 8.3 with acetic acid. The product was extracted into dichloromethane from aqueous solution. The separated organic layer was concentrated and the obtained residue was triturated with dichloromethane and petroleum ether at 10 °C. The separated solid was filtered and dried to give 25 g with 47% of yield.

**Yield:** 41%.

**FT-IR (cm⁻¹):** 3450 (N-H), 2967 (C-H), 1624, 1588 (C=C, C=N), 1565, 1514 (N-H), 1473 (C-H), 1027 (C-O), 1330 (O=S=O), 766, 686 (Aromatic C-H)

**¹H NMR δ ppm:** 2.20 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 3.71 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 5.04 (s, 2H, CH₂), 7.04 (d, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 8.07 (s, 1H, Ar-H), 13.6 (s, N-H).

**Mass:** (m/e): 362(M⁺+H).

**Synthesis of Impurity 7**

A solution of compound 3 (50 g, 0.268 mol) in dichloromethane (250 mL), m-CPBA (92 g, 0.533 mol) dissolved in dichloromethane (100 mL) was added at ambient temperature for 1h then the reaction mass was stirred for 45 min. After completion of the reaction (vide TLC) the reaction mixture was quenched with a solution of sodium hydroxide (15 g) and water (300 ml). The dichloromethane layer was separated and discarded, the aqueous layer was cooled to 0-5 °C and pH was adjusted to 8.3 with acetic acid. The product was extracted into dichloromethane. The separated organic layer was concentrated and the obtained residue was triturated with dichloromethane and petroleum ether at 10 °C. The separated solid was filtered and dried to give 25 g with 47% of yield.
ether at 10 °C. The separated solid was filtered and dried to give the compound 7a 35 g, 78% of yield.

Compound 7a (35 g, 0.21 mol) was condensed with 2 (37.9 g, 0.210 mol) in the presence of sodium hydroxide (21 g, 0.52 mol) in water (200 mL) to give compound 7b with 65 g of 86%.

Compound 7b (50 g, 0.144 mol) in chloroform (250 mL), m-CPBA (25 g, 0.144 mol) dissolved in chloroform (100 mL) and added at -15 °C for 1h then the reaction mass was stirred for 45 min. After completion of the reaction (vide TLC) the reaction mixture was quenched with a solution of sodium hydroxide (15 g) and water (300 mL). The chloroform layer was separated and discarded, the aqueous layer was cooled to 0-5 °C and pH was adjusted to 8.3 with acetic acid. The product was extracted into dichloromethane. The separated organic layer was concentrated and the obtained residue was trituated with dichloromethane and petroleum ether at 10 °C. The separated solid was filtered and dried to give impurity 7 with 23 g of 44%.

**FT-IR (cm⁻¹):** 3440 (N-H), 3060 (C-H), 2939 (C-H), 1626, 1461(C=C, C=N), 1488 (C-H), 1267 (N-O), 1209, 1038 (C-O), 1064 (S=O), 829, 716 (C-H).

**¹H NMR δ ppm:** 2.01 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 3.63 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 4.82 (s, 2H, CH₂), 6.94 (d, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 7.34 (s, 1H, Ar-H), 7.55 (d, 1H, Ar-H).

**Mass:** (m/e): 362(M⁺+H).

**Synthesis of Impurity 8**

Condensation of compound 4 (50 g, 0.15 mol) with compound 3 (28.3 g, 0.15 mol) in the presence of sodium hydroxide (15 g, 0.375 mol) in water (250 mL) to give compound 11 with 55 g of 76%. A solution of compound 11 (50 g,
0.104 mol) in chloroform (250 mL), m-CPBA (18 g, 0.104 mol) was dissolved in chloroform (100 mL) was added at -15 °C for 1h then the reaction mass was stirred for 45 min. After completion of the reaction (vide TLC) the reaction mixture was quenched with a solution of sodium hydroxide (15 g) and water (300 ml). The chloroform is separated and discarded, the aqueous layer was cooled to 0-5 °C and pH was adjusted to 8.3 with acetic acid. The product was extracted into dichloromethane, the separated organic layer was concentrated and the obtained residue was triturated with dichloromethane and petroleum ether at 10 °C. The separated solid was filtered and dried to give 25 g with 49%.

**FT-IR (cm⁻¹):** 3421 (Moisture O-H), 2947 (C-H), 2836 (C-H), 1618, 1587 (C=C, C=N), 1455 (C-H), 1267, 1074 (C-O), 1218 (C-N), 1154 (S=O), 805 (C-H).

**¹H NMR δ ppm:** 2.15 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 3.55 (s, 3H, OCH₃), 3.64 (s, 3H, OCH₃), 3.64 (s, 3H, OCH₃), 4.79 (d, 2H, CH₂), 5.39 (d, 2H, CH₂), 6.80 (m, 1H, Ar-H), 6.97 (m, 1H, Ar-H), 7.23 (m, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H). **Mass:** (m/e): 496 (M⁺+H).

**Synthesis of Impurity 9**

To a solution of impurity 7 (25 g, 0.069 mol) in chloroform (250 mL), m-CPBA (11.9 g, 0.069 mol) dissolved in chloroform (100 mL) was added at -15 °C for 1h, then the reaction mass was stirred for 45 min. After completion of the reaction (vide TLC) the reaction mixture was quenched with a solution of sodium hydroxide (15 g) and water (300 ml). The chloroform was separated and discarded, the resulting mixture was cooled to 0-5 °C and pH was adjusted to 8.3 with acetic acid. The product was extracted into dichloromethane from aqueous solution. The separated organic layer was concentrated and the
obtained residue was triturated with dichloromethane and petroleum ether at 10 °C. The separated solid was filtered and dried to give 15 g with 57%.

**FT-IR (cm⁻¹)**: 3391 (N-H), 3068 (C-H), 2924 (C-H), 1625, 1589 (C=C, C=N), 1481 (C-H), 1268 (N-O), 1203, 1029 (C-O), 1309 (O=S=O), 830, 736 (C-H)

**¹H NMR δ ppm**: 2.10 (s, 3H, CH₃), 2.20 (s, 3H, CH₃), 3.63 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 5.37 (s, 2H, CH₂), 6.94 (d, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 7.55 (d, 1H, Ar-H), 8.05 (s, 1H, Ar-H).

**Mass**: (m/e): 378(M⁺+H).

**Synthesis of Impurity 10**

Condensation of compound 12 (50 g, 0.32 mol) with compound 2 (5 g, 0.31 mol) in sodium hydroxide (32 g, 0.8 mol), water (250 ml) to give compound 13 with 73g (76%). To a solution of compound 13 (50 g, 0.16 mol) in chloroform (250 mL), m-CPBA (28.8 g, 0.16 mol) dissolved in chloroform (100 mL) was added at -15 °C for 1h then the reaction mass was stirred for 45 min. After completion of the reaction (vide TLC) the reaction mixture was quenched with a solution of sodium hydroxide (15 g) in water (300 mL). The chloroform was separated and discarded, the resulting mixture was cooled to 0-5 °C and pH was adjusted to 8.3 with acetic acid. The product was extracted into dichloromethane from aqueous solution. The separated organic layer was concentrated, and the obtained residue was triturated with dichloromethane and petroleum ether at 10 °C. The separated solid was filtered and dried to give impurity 10 with 23 g of 44%.

**FT-IR (cm⁻¹)**: 3433 (N-H), 3050 (C-H), 2989, 2904 (C-H), 1625, 1586 (C=C, C=N), 1409 (C-H), 1204, 1016, (C-O), 1115 (S=O), 822 (C-H).
$^1$H NMR δ ppm: 2.25 (s, 3H, CH$_3$), 2.28 (s, 3H CH$_3$), 3.81 (s, 3H, OCH$_3$), 4.70 (d, 2H, CH$_2$), 6.92 (dd, 1H, Ar-H), 7.10 (d, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H).

Mass: (m/e): 315(M$^+$+H).

4.4 Introduction (Polymorphism of Omeprazole)

Polymorphism is the occurrence of different crystalline forms of a single compound and it is a property of some compound and complexes. Thus, polymorphs are distinct solids sharing the same molecular formula, yet each polymorph may have distinct physical properties. Therefore a single compound may give rise to a variety of polymorphic forms where each form has different and distinct physical properties, such as different solubility profiles, different melting point temperatures and/or different X-ray diffraction peaks. Since the solubility of each polymorph may vary, identifying the existence of pharmaceutical polymorphs is essential for providing pharmaceuticals with predictable solubility profiles. It is desirable to investigate all the solid forms of a drug, including all polymorphic forms, and to determine the stability, dissolution and flow properties of each polymorphic form. Polymorphic forms of a compound can be distinguished in the laboratory by powder X-ray diffraction spectroscopy.$^{10-12}$

The term polymorphism includes different physical forms, crystal forms and crystalline/liquid crystalline/non crystalline (amorphous) forms. It has
been observed that many antibiotics, antibacterials, tranquilizers etc, exhibit polymorphism and some/one of the polymorphic forms of a given drug exhibit superior bioavailability and consequently show much higher activity compared to other polymorphs. For some therapeutic indications one bioavailability pattern may be favored over another. Cefuroxime axetil is the classical example of amorphous form exhibiting higher bioavailability.

In omeprazole free base Karin et al.,\textsuperscript{13} has surprisingly have been found that the substance omeprazole 1 can exist in more than one crystal form and identified a process for the preparation of omeprazole form-A and form-B, substantially free from other forms of omeprazole. The form A is further characterized as being essentially nonhygroscopic in nature. The omeprazole form A is obtained upon slow crystallization which is a crystalline form exhibiting advantageous properties, such as thermodynamically more stable and less hygroscopic than omeprazole form B, especially at room temperature. Omeprazole form A is characterized by the positions and intensities of the peaks in the X-ray powder diffractogram (Table: 4.1).

The peaks, identified with $d$-values calculated from the Bragg formula and intensities, have been extracted from the Guinier diffractogram. Omeprazole form A is prepared by recrystallizing omeprazole of any form, or mixtures of any forms by adding at room temperature to methanol containing catalytic amount of ammonia. The suspension is stirred in darkness for approximately 45 hours and then filtered. The filtrate is dried for 18 hours at 30 °C under reduced pressure.

The Omeprazole form B is unstable, it is obtained upon fast crystallization. Under certain conditions completely or partly it is converted
into omeprazole form A. During crystallization the unstable form is frequently obtained first, then that subsequently transforms into a stable form-A.14

**Table: 4.1: XRD data of form-A**

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The literature shows Omeprazole free base is unstable in the solid state and is susceptible to degradation therefore it can be stabilized in its alkaline salt form.

Omeprazole crystalline form A is patented hence cannot be marketed till the expiry. Omeprazole crystalline form B is not consistent when prepared with the existing process.\textsuperscript{13} Hence the option is to go with the prior art process of omeprazole form B which is unstable.

Here we have got an opportunity for challenging to prepare the stable crystalline omeprazole form B. Hence a comprehensive study is undertaken to synthesize the stable crystalline omeprazole form B and characterize the polymorphism and stability study.

Sherman\textsuperscript{15} reported a process for the preparation of amorphous form of omeprazole magnesium by flash-evaporation. When the magnesium is immersed in the alcohol, the reaction will be evident from the generation of hydrogen bubbles, and the reaction will complete when all the magnesium has been consumed and the effervescence has ceased. The total magnesium will be present as magnesium alkoxide. The omeprazole can then add directly to the magnesium alkoxide solution. In order to obtain solid magnesium omeprazole that is substantially free of organic solvent, it is then necessary to eliminate the complete solvent. It can be done by using "flash-evaporating", to mean evaporating in such a way as to avoid the precipitation of crystals or large particles which entrap the alcohol.

\textbf{4.4.1 Present work}
We have studied for the preparation of stable crystalline omeprazole form B with a novel process which involves using agitated thin film dryer (ATFD).

In this process the omeprazole of any form is dissolved in mixture of solvents and concentrated under vacuum in agitated thin film dryer at lower temperature and the product is taken out from bottom of the reactor. The product is confirming the polymorphism from powder X-ray diffraction spectroscopy data (PXRD).

Omeprazole form A is obtained by reaction crystallization or recrystallizing omeprazole of any form or mixtures of any forms, in an appropriate solvent such as methanol at room temperature for prolonged time period of 2 h to several weeks.\textsuperscript{13}

\subsection*{4.4.1.1 Preparation of stable omeprazole form B}

Omeprazole form B prepared\textsuperscript{13} by reaction crystallization of omeprazole in methanol with ammonia as catalyst at 50 °C. The solution is cooled to 0 °C in 20 minutes. The formed crystals were filtered, washed with ice-cooled methanol and subsequent drying gives the omeprazole form B. Omeprazole form B prepared according to this process is not consistent. This process is not suitable to prepare omeprazole form B in commercial scale with consistency.

There is a constant need to prepare pharmaceutically stable crystalline form of the active substance omeprazole in an industrially simple and readily feasible way with high yield and quality. This makes us to attempt on different studies to prepare stable crystalline omeprazole form B.

Omeprazole form-B is prepared upon fast crystallization, based on this concept the following process strategies are followed to identify the process for
preparation of stable crystalline omeprazole form-B. Conducted experimental study with the following processes.

I. Omeprazole of any form is dissolved in methylene chloride and neutralized with different anti solvents at different temperatures then stirred for solid separation and filtered the solid immediately.

II. Omeprazole of any form is dissolved in methylene chloride and neutralized with acetone at different temperatures then stirred for solid separation and filtered the solid immediately.

III. Stirring$^{13}$ a mixture of omeprazole of any form in methanol and ammonia at 50-55 °C for 10-15 minutes. Cooled the reaction mass to 0 °C in 20-30 minutes for solid separation and filtered the solid immediately.

IV. Stirring$^{16}$ a mixture of omeprazole of any form in aqueous basic methanol for clear solution. Cooled to 0-5 °C, then adjusted the pH to 8.2 with acetic acid for solid separation and filtered the solid immediately.

V. Omeprazole of any form with catalytic amount of water is dissolved in methylene chloride and neutralized with acetone at different temperatures for solid separation and filtered the separated solid immediately. With this process by using water in the reaction, the omeprazole form B obtained is consistent to some extent, but filtration has to be done immediately, after solid separation.

From all the above process it is understood that the above methods are not suitable for preparation of stable omeprazole form B in commercial scale with consistency, but two things are understood from this experimental study
that the omeprazole of any form dissolving in methylene chloride with an amount of water and neutralizing with anti solvent for solid separation at lower temperatures, followed by the filtration of the solid immediately. The solid prepared from this process is somewhat consistent for preparing omeprazole form B, but this procedure is not suitable in commercial scale, since the filtration of the product has to be done immediately. Therefore based on the above study, assuming that the omeprazole by dissolving in solvent or mixture of solvents with an amount of water and evaporating the solvent in agitated thin film drier (ATFD) under vacuum, the product obtained might be stable crystalline omeprazole form B. Hence drawn the graph to dissolve the omeprazole in different solvents and mixture of solvents. Amusingly the above sketch drives to prepare the stable crystalline omeprazole form B.

We encountered to prepare the stable crystalline omeprazole form B consistently in commercial scale with a technique called ATFD. ATFD is used to convert liquids, slurries and pastes to free-flowing solids in continuous, single-pass operation. Agitated thin film dryers have a short residence time and are especially useful for processing heat sensitive products, due to low ‘hold-up’ and self-cleaning heating surfaces.

In this process omeprazole of any form is dissolved in mixture of solvents methylene chloride, methanol and water, followed by feeding of the solution into moving hinged blades spread over a heated wall. The thickness of the layer is defined by the clearance between the blade and the heated wall. A highly agitated bow wave is formed in front of the rotating blades. The turbulence increases as the product passes through the clearance before entering a calming zone situated behind the blades. The volatile component evaporates
continuously under vacuum (600-700 mm/Hg). The product layer is a few millimeters in thickness. The hinged pendulum blades are designed to give a minimum clearance with the dryer wall to prevent fouling of the heating surface by product. However, the blades do not themselves contact the heated wall. The solid obtained is pushed to the receiver where the solid can be taken out. (Diagram: 4.1).

The brief description of the process involves omeprazole of any form dissolved in mixture of solvents methylene chloride, methanol and water, followed by passing the reaction mass from the injector, where the sample is injected 1 L for half-an-hour and the solvent is evaporated completely under high vacuum. The separated solid is pushed to the receiver where the solid can be taken out from bottom of the reactor and subsequent drying gives the stable crystalline omeprazole form B. Omeprazole form B obtained by this process is consistent and this process is validated in commercial scale. The differential scanning calorimetry thermogram exhibits a significant endo-exo pattern respectively at 157.5 °C and 160 °C. Powder X-Ray diffractogram (PXRD) reveals that characteristic form A peak is below LOD (5% in form B).

4.4.1.2 Stability study

Further to understand the stability of the obtained omeprazole form B, samples were examined against air, humidity, stress and cold stability conditions. Omeprazole form B when estimated for water content by karl fisher's reagent shows the presence of water content less than 0.5%. Hence the omeprazole form B prepared is anhydrous crystalline. The hygroscopic study was conducted for omeprazole form B, samples kept at 25-30 °C under open-air condition and 90% relative humidity, the results was tabulated below.
a) Sample kept at 25-30 °C with open-air exposure

Water content of the sample increases by about 0.14% from initial to 7th day when exposed to open air. There is no significant change in the IR spectrum and powder XRD diffractogram (Table: 4.2).

Table: 4.2

<table>
<thead>
<tr>
<th>Stability study &amp; storage condition</th>
<th>Testing intervals</th>
<th>Water content (%)</th>
<th>IR</th>
<th>XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample-1 of form-B exposed to air at 25-30 °C.</td>
<td>Initial</td>
<td>0.24</td>
<td>Matching with standard</td>
<td>Matching with standard</td>
</tr>
<tr>
<td></td>
<td>1st day</td>
<td>0.27</td>
<td>Matching with standard</td>
<td>Matching with standard</td>
</tr>
<tr>
<td></td>
<td>3rd day</td>
<td>0.37</td>
<td>Matching with standard</td>
<td>Matching with standard</td>
</tr>
<tr>
<td></td>
<td>7th day</td>
<td>0.38</td>
<td>Matching with standard</td>
<td>Matching with standard</td>
</tr>
</tbody>
</table>

b) Sample kept at 90% relative humidity

Water content of the sample increases by about 0.25% from initial to 7th day when exposed to 90% relative humidity. There is no significant change in the IR spectrum and powder XRD diffractogram. Based on the observations it is concluded omeprazole form B is very slightly hygroscopic in nature.

Table: 4.3

<table>
<thead>
<tr>
<th>Stability study &amp; storage condition</th>
<th>Testing intervals</th>
<th>Water content (%)</th>
<th>IR</th>
<th>XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample-1 of form-B</td>
<td>Initial</td>
<td>0.24</td>
<td>Matching with standard</td>
<td>Matching with standard</td>
</tr>
</tbody>
</table>
meprazole form B under stress stability condition

Description was changed in thermal and compression conditions. In related substance by HPLC, one unknown impurity is observed only in photo stability study.

In all the above samples PXRD diffractgram, form A content is below limit of detection (LOD of form-A is 5%).

Table: 4.4

<table>
<thead>
<tr>
<th>S. No</th>
<th>Stress conditions</th>
<th>Duration</th>
<th>Description</th>
<th>I.R. spectrum</th>
<th>Water content</th>
<th>Purity by HPLC</th>
<th>XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Accelerated stability (40 °C &amp; 75% Relative humidity)</td>
<td>24 h</td>
<td>Off-white crystalline powder</td>
<td>Complies</td>
<td>0.28</td>
<td>99.93</td>
<td>No significant change</td>
</tr>
<tr>
<td>2.</td>
<td>Thermal (90 °C)</td>
<td>24 h</td>
<td>Light cream crystalline powder</td>
<td>Complies</td>
<td>0.21</td>
<td>99.94</td>
<td>No significant change</td>
</tr>
<tr>
<td>3.</td>
<td>Photo stability (UV)</td>
<td>24 h</td>
<td>Off-white crystalline powder</td>
<td>Complies</td>
<td>0.16</td>
<td>99.85</td>
<td>No significant change</td>
</tr>
</tbody>
</table>
Omeprazole form B in cold storage

B sample packed in clear polyethylene bag filled with nitrogen, tied with tag and kept in a black polyethylene bag along with silica pouch filled with nitrogen and then sealed. Finally the sample kept in triple laminated bag along with silica pouch, sealed and stored in HDPE drum. There is no significant change observed during the study. Sample is stable for three months at cold storage stability conditions.

<table>
<thead>
<tr>
<th></th>
<th>4. Compress ion (2000 kg/cm²) for 1 h.</th>
<th>1 h</th>
<th>Light gray crystalline powder</th>
<th>Complies</th>
<th>0.22</th>
<th>99.93</th>
<th>No significant change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table: 4.5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration</th>
<th>Description</th>
<th>LOD</th>
<th>Identification by IR</th>
<th>PXRD</th>
<th>Purity by HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Off-white colored powder</td>
<td>0.21</td>
<td>Complies</td>
<td>Complies</td>
<td>99.92</td>
</tr>
<tr>
<td>1st Month</td>
<td>Off-white colored powder</td>
<td>0.24</td>
<td>Complies</td>
<td>Complies</td>
<td>99.94</td>
</tr>
<tr>
<td>2nd month</td>
<td>Off-white colored powder</td>
<td>0.23</td>
<td>Complies</td>
<td>Complies</td>
<td>99.93</td>
</tr>
<tr>
<td>3rd Month</td>
<td>Off-white colored powder</td>
<td>0.25</td>
<td>Complies</td>
<td>Complies</td>
<td>99.93</td>
</tr>
</tbody>
</table>
Stable omeprazole form B synthesized consistently in commercial scale by isolating the solid using a technique called agitated thin film drier.

Diagram: 4.1 Agitated thin film dryer (ATFD)
Fig: 4.11 The comparison of four crystalline samples of omeprazole form B with 5% of omeprazole form A.
4.4.2 Amorphous form of Omeprazole magnesium

Introduction

The classical method for purification used is crystallization from solution of pharmaceutically active compounds or their intermediates. The nature of the crystal arrangement, or crystal pattern, that is produced and the particle size of the end product are important consideration in the crystallization process.

High bioavailability and short dissolution time are desirable or often necessary attributes of the pharmaceutical end product. However, the direct crystallization of small sized, high surface area particles is usually accomplished in a high super saturation environment which often results in material of low purity, high friability and decreased stability due to poor crystal structure formation. Because the bonding forces in organic crystal lattices generate a much higher frequency of amorphous that those found in highly ionic inorganic solids, oiling out of supersaturated material is not uncommon, and such oils often solidify without structure.\(^{18}\)

Currently, pharmaceutical compounds almost always require a post-crystallization milling step to increase particle surface area and thereby improve their bioavailability. However, high energy milling has drawbacks. Milling may result in yield loss, noise and dusting, as well as unwanted personnel exposure to highly potent pharmaceutical compounds. Also, stresses generated on crystal surfaces during milling can adversely affect labile compounds.

When using current reverse addition technology for direct small particle crystallization, a concentration gradient cannot be avoided during initial crystal formation because the introduction of feed solution to anti-solvent in the
stirred vessel does not afford a thorough mixing of the two fluids prior to crystal formation. The existence of concentration gradients, and therefore a heterogeneous fluid environment at the point of initial crystal formation, impedes optimum crystal structure formation and increases impurity entrainment. If a slow crystallization technique is employed, more thorough mixing of the fluids can be attained prior to crystal formation which will improve crystal structure and purity, but the crystals produced will be large and milling will be necessary to meet bioavailability requirements.

Another standard crystallization procedure employs temperature variation of a solution of the material to be crystallized in order to bring the solution to its super saturation point, but this is a slow process that produces large crystals. Also, despite the elimination of a solvent gradient with this procedure, the resulting crystal characteristics of size, purity and stability are difficult to control and are inconsistent from batch to batch.

Now with the amorphous polymorph eliminates the need for subsequent high intensity milling to meet bioavailability requirements. By removing the need for milling, the amorphous process avoids associated problems of noise and dusting, cuts the yield loss and saves the time and extra expense incurred during milling. It also removes an extra opportunity for personnel contact with a highly potent pharmaceutical agent, or for adverse effects on labile compounds. The small particle size attained with the amorphous is consistent within a single run and results are reproducible between runs.

Amorphous form is one of the polymorphism in almost all the prazoles. The value of amorphous form is high due to its high solubility than the crystalline form and due to the patents on crystalline polymorphs. During the
process development of omeprazole targeted to prepare amorphous form which can make full-manufacturing scale.

4.4.3 Present work

There is a requirement to put efforts for the development of a commercial process to make amorphous form of 13. Initially the amorphous form is prepared as follows:

a. reacting the omeprazole with magnesium alkoxide in presence of alcohol.

b. mixing the reaction mass properly for uniformity.

c. evaporating the alcohol completely.

d. isolation in non-polar solvents, with this usual isolation process the amorphous form cannot be isolated at commercial scale due to its gummy nature.

At the time of isolation the compound is in contact with solvent for more time hence the solid obtained is not amorphous consistently. To overcome this problem a comprehensive study was undertaken to synthesize the amorphous form in commercial scale.

The amorphous form in omeprazole magnesium 13, is prepared in commercial scale by using agitated thin film dryer (ATFD)\(^2\) which can be isolated easily without any difficulties. In this technique the compound can be isolated with high yield by way of consistency, since there is no isolation point by using solvent.

Agitated thin film dryer are used to convert liquids, slurries, and pastes to free-flowing solids in continuous, single-pass operation. Agitated thin film
Dryers have a short residence time and are especially useful for processing heat sensitive products, due to low ‘hold-up’ and self-cleaning heating surfaces.

This technique is used in commercial scale for preparation of amorphous polymorph of omeprazole magnesium (13). In this process a mixture of magnesium, methanol and methylene chloride were stirred for dissolution of magnesium at 55-60 °C. The reaction mass was cooled to room temperature and added omeprazole then stirred for dissolution. The reaction mass was filtered through hyflow for particle free, and feed the solution into moving hinged blades spread over a heated wall. The thickness of the layer is defined by the clearance between the blade and the heated wall. A highly agitated blow wave is formed in front of the rotating blades. The turbulence increases as the product passes through the clearance before entering a calming zone situated behind the blades. The volatile component evaporates continuously under vacuum (600-700 mm/Hg). The product layer is a few millimeters in thickness. The hinged pendulum blades are designed to give a minimum clearance with the dryer wall to prevent fouling of the heating surface by product. However, the blades do not themselves contact the heated wall. The solid obtained is pushed to the receiver where the solid can be taken out as amorphous form consistently (ATFD Diagram 4.1).

The process for the preparation of said amorphous form uses conditions which are convenient to perform on a commercial scale and operationally safe. The product is confirming the polymorph from powder X-ray diffraction data.
4.5 Experimental section

4.5.1 Preparation of omeprazole form-B

A mixture of dichloromethane (112 L) and methanol (30 L) was stirred at 5 °C for 10 minutes and added omeprazole (15 kg). The reaction mass was then stirred about 10 minutes, to which was then added water (30 L) and stirred about 10 minutes to get a clear solution. The reaction mass was then transferred into a feed tank of the agitated thin film drier by applying cooling temperature of about 5 °C, followed by passing the reaction mass from the injector, where the sample was injected 1 L for half-an-hour and the solvent was evaporated completely under high vacuum. The solid that obtained was then fed into a microniser under nitrogen atmosphere pressure between 2.5-3.5 Kg/cm², and then transferred into a fluid bed drier for further drying of the material at a temperature of about 40 to 45 °C to afford the stable omeprazole form B.

4.5.2 Preparation of Omeprazole Magnesium (13)

A mixture of magnesium (173.9 g, 7.24 mol), methanol (7.5 L) and methylene chloride (300 ml) were stirred for dissolution of magnesium at 55-60 °C for 3 h. The reaction mass was cooled to 20 °C and followed by methanol (67.5 L) and omeprazole (5 Kg, 14.49 mol) was added and stirred for 1 h at 20 °C. The reaction mass was filtered through hyflow for particle free, and passed through agitated thin film dryer at 40 °C under vacuum of 600-700 mm/Hg. The separated solid was collected from the reactor, (4.91 Kg, 95%).

4.6 References


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**CHAPTER-V**