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Structural identification and characterization of potential impurities of rabeprazole sodium

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ABSTRACT

Rabeprazole sodium (Aciphex) is a gastric proton pump inhibitor used for the prevention and treatment of gastric acid related diseases. During the synthesis of bulk drug of rabeprazole sodium we observed seven impurities, in which four are new impurities. All the impurities were detected by a gradient high performance liquid chromatographic (HPLC) method, whose area percentages were ranged from 0.05 to 1.0%. LCMS was performed to identify the mass number of these impurities. A thorough study was carried out to characterize the impurities. These impurities were synthesized, characterized and were co-injected with the sample containing impurities and are found to be matching with the impurities present in the sample. Based on the complete spectral analysis (IR, NMR and Mass) these impurities were characterized as Rabeprazole sulphone [Impurity:1]; Rabeprazole sulphide [Impurity:3]; 1H- benzimidazole-2-thiol [Impurity:5]; 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]-1H-benzimidazole [Impurity:6]; 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfonyl]-1H-benzimidazole [Impurity:7]; 5-[1H-benzo[d]imidazol-2-ylthio]-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylthio]-1H-benzo[d]imidazole [Impurity:8]; 1-[[1H-benzo[d]imidazol-2-ylthio][4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylthio]-1H-benzo[d]imidazole [Impurity:11]. Of which impurities 6, 7, 8 and 11 are new impurities.

Keywords: Rabeprazole; Impurities; Isolation; Preparative High performance liquid chromatography; Characterization.

INTRODUCTION

Rabeprazole, chemically known as (±) 2-[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole sodium salt is a proton pump inhibitor and inhibits the action of H^+-K^+ ATPase in parietal cells [1-5]. Preclinical studies indicated that, rabeprazole is 6.5 times more potent than omeprazole in inhibiting the enzyme activity of isolated gastric vesicles [6] and is an effective drug in the treatment of peptic ulcer. It has also demonstrated efficacy in healing and symptom relief of gastric and duodenal ulcers, as well as a high-eradication rate of the microorganism *Helicobacter pylori* when associated with antimicrobial therapy [7]. The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products.

During the analysis of laboratory batches of rabeprazole sodium, seven impurities are observed in HPLC method. In order to commercialize an API, it is a mandatory requirement by regulatory authorities to identify and characterize all the unknown impurities that are present in it at a level more than 0.1% [8]. These impurities are required in pure form to check the HPLC method performance such as specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, system suitability testing, and relative response factor (RRF) [9,10]. These related substances are also used to check the accuracy of the analytical method of API.

The structure of possible impurities related to process/Raw materials/degradants is identified/characterized by the various characterization techniques such as UV, IR, NMR & Mass and chromatographically by HPLC spiking studies. The pathway for the formation of these impurities is also delineated. Among these seven impurities (Fig.2), impurities 6, 7, 8 and 11 are hitherto not reported, impurity 1 and 3 were known as metabolites [2, 11], and impurity 5 reported as raw material impurity.

In our manufacturing process of rabeprazole sodium (Fig.3), we have identified following seven impurities 2-[[[4-(3-methoxy propoxy)-3-methyl pyridin-2-yl]methyl] sulfonyl]-1-*H*-benzimidazole[Impurity: **1**]; 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]thio]-1-*H*-benzimidazole [Impurity: **3**]; 1H- benzimidazole-2-thiol [Impurity:**5**]; 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]-1H-benzimidazole [Impurity: **6**]; 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfonyl]-1H-benzimidazole [Impurity: **7**]; 5-[1H-benzo[d]imidazol-2-ylthio]-2-[[4-(3-methoxy propoxy)-3-methylpyridin-2-yl]methyl thio]-1H-benzo[d] imidazole [Impurity: **8**]; 1-[[1H-benzo[d]imidazol-2-ylthio][4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylthio]-1H-benzo[d]imidazole [Impurity: **11**]. Rabeprazole Impurity **1** and Impurity **3** are reported as metabolites [2, 11]. An increasing number of publications on development of analytical methods for rabeprazole bulk drug analysis indicate the significance of impurities of rabeprazole [12-21]. In our present investigation, we have taken up the synthesis and characterization of Impurities 6, 7, 8 and 11.

HPLC chromatogram of Rabeprazole Sodium spiked with impurities

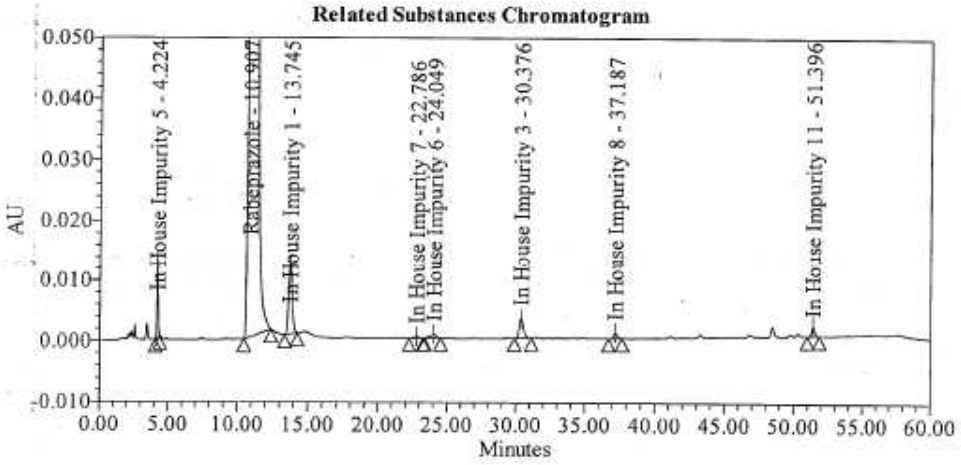


Figure-1

EXPERIMENTAL SECTION

(i) Samples and chemicals

Samples of rabeprazole sodium (Batch No. 846/RBZ-III/124), bulk material was obtained from the R& D department, NPNC division, Orchid Chemicals and Pharmaceutical Limited, Chennai, India. All the seven impurities were synthesized in the laboratory after identification by HPLC and determination of mass number by LC-MS, HPLC grade acetonitrile and acetic acid were obtained from Merck, India. Ammonium acetate, phosphoric acid and triethylamine AR grade were obtained from SD Fine chemicals limited, India. Water used for preparing mobile phase was purified using Millipore Milli-Q plus (Milford, MA, USA) purification system. Chloroform-*d* and dimethylsulphoxide-d_6 were purchased from Euriso-top SA, France.

(ii) High Performance liquid chromatography (HPLC)
An In-house LC gradient method was developed for the analysis of rabeprazole sodium and its impurities (Water Alliance 2695 separations module & Waters 2487 Dual absorbance detector, with Empower software) using a stainless steel column Prontosil Kromabond 100-5-C18, (250X4.6)mm, 5µ with a mobile phase consisting of 0.01 M KH_2PO_4, pH adjusted to 6.6-7.0 with Triethylamine as mobile phase-A. Mobile phase-B was used as a mixture of Acetonitrile and methanol in the ratio 95:5. Composition of eluent was varied at a constant flow rate 1.0 mL/min and UV detection at 280 nm was used. This LC method was able to separate all these impurities.

(iii) Liquid chromatography-mass spectrometry (LC-MS)
Mass spectrometry compatible chromatographic method was developed for the analysis of rabeprazole sodium and its impurities, where a column Prontosil Kromabond 100-5-C18, (250X4.6)mm, with a mobile phase consisting of 0.01 M ammonium acetate, acetonitrile and methanol in the gradient method, with a flow rate of 1.0 ml/min, UV detection at 280 nm was used and the column was kept at 30°C. This LC method was able to separate all these impurities. The mass spectra of impurities were recorded in API-3000 LC-MS/MS mass spectrometer.

(iv) Mass spectrometry
The LC-MS analysis has been performed on API-3000 LC-MS/MS mass spectrometer [PE Sciex, Foster City, CA]. The analysis was performed in both ionization modes with Turbo Ion spray interface with the following conditions. Ion source voltage 5500 V, declustering potential 80 V, Focusing potential 150 V, entrance potential 10 V, with the nebulizer gas as nitrogen at 60 psi were used for positive ionization mode. Whereas the negative ionization was performed by switching the polarity of the ion source voltage to -4500 V.

(v) NMR spectroscopy
The ^{1}H NMR and ^{13}C NMR experiments for rabeprazole sodium impurities were performed at 400.13 MHz and 100.62 MHz respectively on Bruker Avance 400 MHz FT NMR spectrometer with multinuclear BBO probe. DMSO-d_6 and $CDCl_3$ were used as solvents. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.0 ppm) and in the ^{13}C NMR the chemical shift values were reported relative to $CDCl_3$ (δ = 77.0 ppm) and DMSO-d_6 (δ = 39.50 ppm) as reference. COSY, HSQC and HMBC experiments were performed to assign the signals unequivocally. DEPT spectra revealed the presence of methyl and methine groups as positive peaks and methylene as negative peaks.

(vi) Melting points determination
Melting points of all the impurities were determined by using the capillary method on a POLMON digital melting point apparatus Model no. Buchi T1.0AL

(vii) FT-IR spectroscopy
The IR spectra were recorded in the solid state as KBr dispersion medium using FT-IR (Perkin Elmer, Spectrum 65 & JASCO-FT-IR-430) spectrophotometer.

(viii) Synthesis of impurities
Impurity **3** 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]thio]-1-*H*-benzimidazole [Rabeprazole Sulfide] is the precursor of rabeprazole. NaOCl oxidation of **3** results rabeprazole. Rabeprazole on further oxidation with NaOCl forms Impurity **1** 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfonyl]-1-*H*-benzimidazole [Sulphone] and Impurity **6** 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]-1H-benzimidazole is formed due to the chlorination with the nascent chlorine present in NaOCl. Impurity **7** 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfonyl]-1H-benzimidazole is formed due to chlorination of Sulphone[Impurity **1**] with the nascent chlorine present in NaOCl. Impurity **8** 5-[1H-Benzo[d]imidazol-2-ylthio]-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylthio] -1H-benzo[d]imidazole is formed due to the reaction of 5-Chloro-2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfonyl]-1H-benzimidazole with 1H- benzimidazole-2-thiol [Impurity: 5]. Chlorination of Impurity **3** with nascent chlorine present in the NaOCl followed by the reaction with Impurity **3** itself forms Impurity **11.**

*Preparation of impurities **6** and **7***
Sodium hypochlorite (28 ml, 0.5 eq.) and sodium bicarbonate solution (2.4 g, 2eq. in 6.0 ml water) were added to the mixture of IPA (15 ml) and rabeprazole sulphide (5g, Impurity **3**) at 0-5°C, then 0.8 equivalents (In three intervals of 28 ml, 8.4 ml and 8.4 ml) of NaOCl was added at the interval of 1hr each at 0-5°C. Reaction was

continued for 14 hrs at 0-5°C.Then it was quenched with sodium thiosulphate solution (5.2g in 10ml water) and charged 17ml water at 0-5°C. Reaction mass was treated with carbon (10%w/w). Charged Dichloromethane (10 ml) and adjusted the pH to 8-8.5 using formic acid below 20°C. Aqueous layer was extracted with Dichloromethane (2x10ml). Dichloromethane was distilled out completely to get residue. Above residue gave impurities **6** and **7** by preparative HPLC.

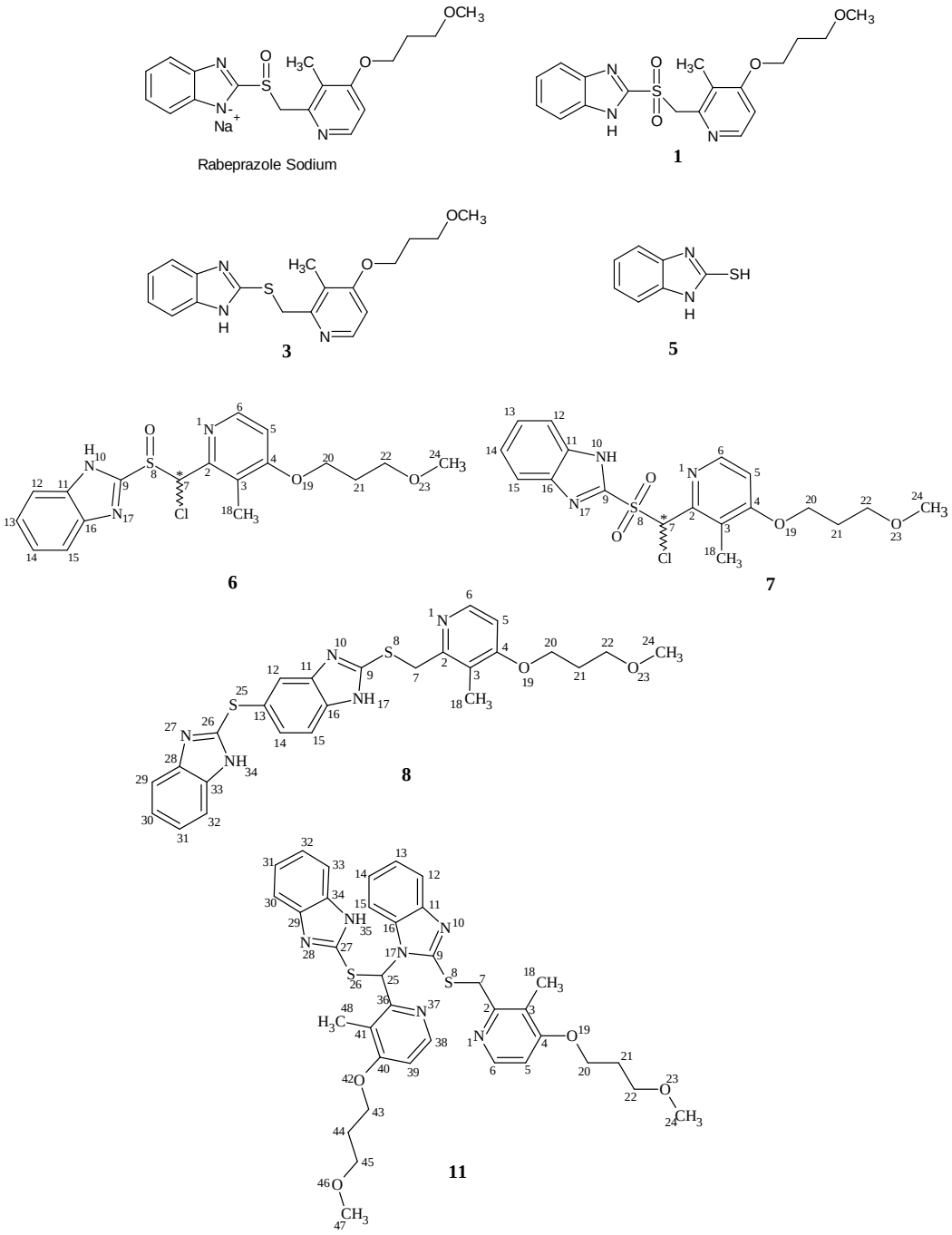


Fig.2. Atom numbering used for NMR assignment

*Preparation of Impurities **8** and **11***

Rabeprazole free base (50 g) was dissolved in ethyl acetate (400 ml) at 50-55°C and added diethyl amine (6.4 ml, 0.45 eq). Reaction mixture was cooled to 25-30°C. Reaction mixture was further cooled to 0-5 °C, and maintained at this temperature for 5 hr. Solid formed was filtered and washed with chilled ethyl acetate (2x50 ml). Distilled out ethyl acetate completely to get gummy residue. Impurities **8** and **11** were isolated by preparative HPLC from the above residue**.**

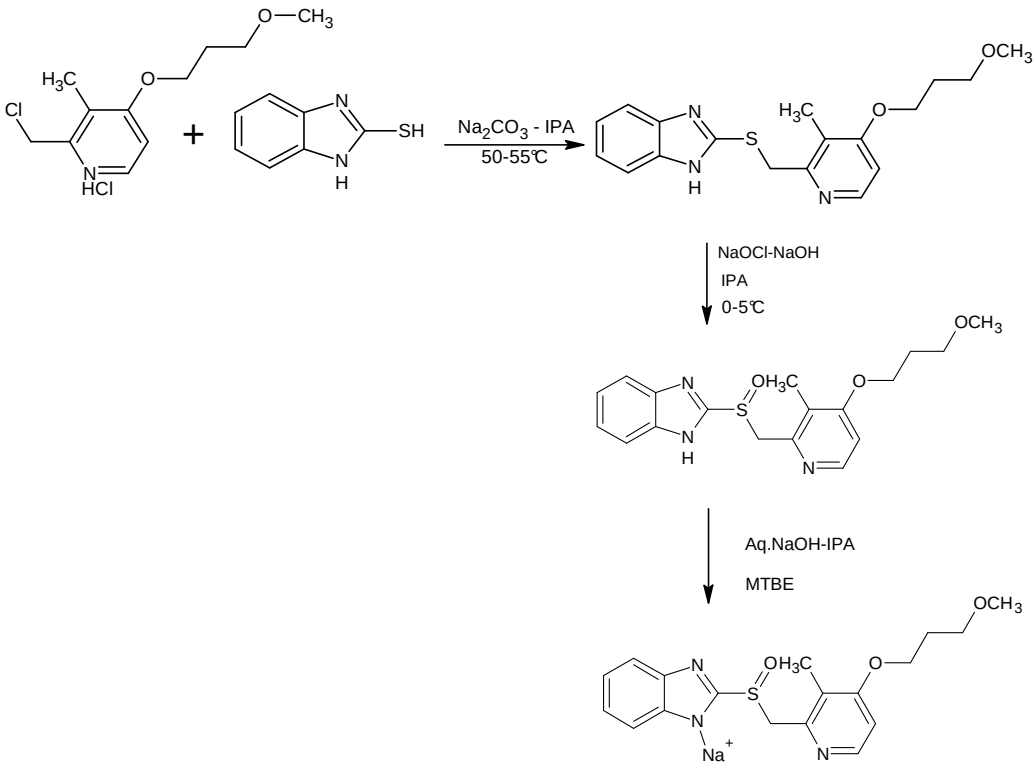


Fig.3. Scheme for the synthesis of rabeprazole sodium

Table.1: **The relative retention time of known eluting peaks with respect to rabeprazole peaks are given below**

S. No.	RRT	Name of Impurity	Nature of impurity
1	0.39	*Rabeprazole sodium Impurity-5*	KSM
2	1.26	*Rabeprazole sodium Impurity-1*	Degradation
3	2.09	*Rabeprazole sodium Impurity-7*	Process Related
4	2.21	*Rabeprazole sodium Impurity-6*	Process Related
5	2.79	*Rabeprazole sodium Impurity-3*	Process Related
6	3.41	*Rabeprazole sodium Impurity-8*	Process Related
7	4.71	*Rabeprazole sodium Impurity-11*	Process Related
8	**1.00**	**RABEPRAZOLE SODIUM**	

RESULTS

A typical analytical HPLC chromatogram of laboratory batch of Rabeprazole sodium bulk drug recorded using the LC method is described in section 2.2. The target impurities under study are marked as impurity 6 (retention time (RT): 24.04, molecular weight (MW): 394), impurity 7 (RT: 22.78, MW: 410), impurity 8 (RT: 37.18, MW: 492),

impurity 11 (RT: 51.39, MW: 685). The LC-MS compatible method which is used to detect the impurities is described in section 2.3 which is used to detect all the impurities Fig.1. RRT and name of these impurities and rabeprazole sodium are shown in Table 1. All impurities (6, 7, 8 and 11) are non-polar relative to the Rabeprazole Sodium.

Melting range, FT-IR and Mass spectral data of Impurities

Compound	M.pt (°C)	IR	MS
Impurity 7	Stable upto 285°C.	3442 (-NH stretching), 2923 (Asymmetric-CH2 stretching) 1585 (Aromatic –C=C & -C=N stretching) 900-675 (Out of plane –CH bending)	+ve mode m/z: 410 [M+H]+, 412 Chlorine isotopic peak, 473 [M+Na(CH3CN)]+
Impurity 6	140-142°C	3432 (-NH stretching), 2925(Asymmetric-CH2 stretching) 1585 (Aromatic –C=C & -C=N stretching) 900-675 (Out of plane –CH bending)	+ve mode m/z: 394 [M+H]+ , 396 Chlorine isotopic peak, 416 [M+Na]+
Impurity 8	133.5-134°C	3435 (-NH stretching), 2924(Asymmetric-CH2 stretching) 1600-1400 (Aromatic –C=C & -C=N stretching) 900-675 (Out of plane –CH bending)	+ve mode m/z: 492 [M+H]+, 493 isotopic peak of 492.
Impurity 11	75-80°C	3435 (-NH stretching), 2924(Asymmetric-CH2 stretching) 1600-1400 (Aromatic –C=C & -C=N stretching) 900-675 (Out of plane –CH bending	+ve mode m/z: 685 [M+H]+, 686 isotopic peak of 685.

DISCUSSION

The positive electro spray ionization (ESI) mass spectrum showed the *m/z* peaks at 394 $[M+H]^+$, 396 $[M+2]^+$ and 416 $[M+Na]^+$ which is indicative of one chlorine atom present in the molecule. IR spectrum displayed characteristic absorptions at 3432, 2925, 1585 and 900-675 cm^{-1} which is indicative of NH stretching, asymmetric CH_2 stretching aromatic –C=C-, -C=N- stretching, and ether functionality which is supported by quaternary carbon signals in ^{13}C and DEPT spectrum. ^{13}C NMR is accounted for 18 carbons and DEPT spectrum displayed three negative and nine positive which includes two methyl and seven methine groups, and six carbons considered as quaternary. Based on the above spectral data the molecular formula of Impurity-**6** could be $C_{18}H_{20}ClN_3O_3S$. This molecular formula matched well with the protonated molecular ion observed at *m/z* 394 in the mass spectral data. The data obtained from the spectral studies can be rationalized in terms of Impurity **6** having the molecular formula $C_{18}H_{20}ClN_3O_3S$ and corresponding structure was characterized as 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]-1H-benzimidazole (RRT =2.11)

The mass spectra of the Impurity **7** (RRT=1.96) displayed protonated molecular ion at *m/z* = 410 [M+H], which is 16 amu more than that of Impurity **6** (Protonated molecular ion *m/z* 394). The chemical shift of methine carbon adjacent to sulphur appeared 69.4 and 71.2 ppm for Impurity 6 and Impurity 7 respectively. Thus the Impurity 7 structure can be explained in terms of the addition of oxygen on sulphur in Impurity 6. Based on the above spectral data the molecular formula of Impurity-**7** could be $C_{18}H_{20}ClN_3O_4S$ and corresponding structure was characterized as 2-[[chloro[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfonyl]-1H-benzimidazole (RRT =1.96).

The positive electro spray ionization (ESI) mass spectrum showed the *m/z* peaks at 492 $[M+H]^+$, 493 is the isotopic peak of mass 492. IR spectrum displayed characteristic absorptions at 3435, 2924, 1600-1400 and 900-675 cm^{-1} which is indicative of NH stretching, asymmetric CH_2 stretching aromatic –C=C-, -C=N- stretching, and ether functionality which is supported by quaternary carbon signals in ^{13}C and DEPT spectrum. ^{13}C NMR is accounted for 25 carbons and DEPT spectrum displayed four negative and eleven positive which includes two methyl and nine methine groups, and ten quaternary carbons. 2D NMR data confirms the substitution of MBI to the Impurity-3 at 13^{th} position.

Based on the above spectral data the molecular formula of Impurity-8 could be $C_{25}H_{25}N_5O_2S_2$. This molecular formula matched well with the protonated molecular ion observed at *m/z* 492 in the mass spectral data. The above data obtained from the spectral studies can be rationalized in terms of Impurity **8** is having the molecular formula $C_{25}H_{25}N_5O_2S_2$ and corresponding structure was characterized as 5-[1H-Benzo[d]imidazol-2-ylthio]-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl thio]-1H-benzo[d]imidazole (RRT =3.46)

*^{1}H and ^{13}C NMR assignment for **Impurity 6** and **Impurity 7***

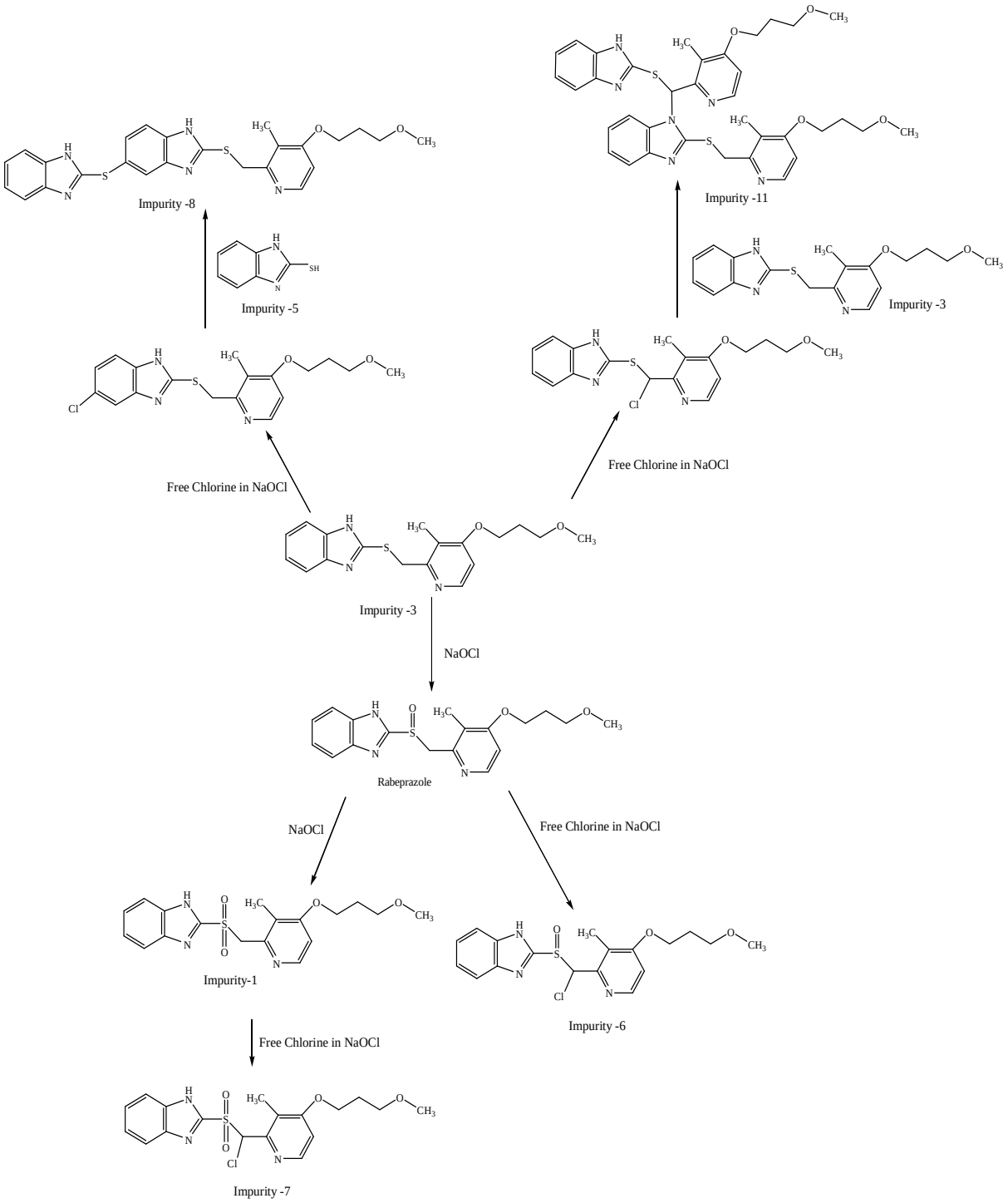
	Impurity 6				***Impurity 7***			
Position	^{1}H	*ppm/J*	^{13}C	*DEPT*	^{1}H	*ppm/J*	^{13}C	*DEPT*
1	-	-			-	-		
2	-	-	*153.1*		-	-	*147.4*	
3	-	-	*123.4*		-	-	*123.5*	
4	-	-	*163.4*		-	-	*162.8*	
5	*1*	*7.13/d,5.6*	*107.8*	*CH*	*1*	*7.06/d,5.6*	*107.7*	*CH*
6	*1*	*8.49/d,5.6*	*148.9*	*CH*	*1*	*8.29/d,5.6*	*148.4*	*CH*
7	*1*	*6.79/s*	*69.4*	*CH*	*1*	*7.11/s*	*71.2*	*CH*
8	-	-			-	-		
9	-	-	*150.4*	*C=N*	-	-	*140.5*	*C=N*
10	-	-			-	-		
11	-	-	*139.2*		-	-		
12	*1*	*7.34/m*	*123.6*	*CH*	*1*	*7.30/m*	*123.3*	*CH*
13	*1*	*7.74/m*	*116.6*	*CH*	*1*	*7.67/m*	*117.4*	*CH*
14	*1*	*7.74/m*	*116.6*	*CH*	*1*	*7.67/m*	*117.4*	*CH*
15	*1*	*7.34/m*	*123.6*	*CH*	*1*	*7.30/m*	*123.3*	*CH*
16	-	-	*139.2*		-	-		
17	-	-			-	-		
18	*3*	*2.32/s*	*10.2*	*CH3*	*3*	*2.3/s*	*10.6*	*CH3*
19	-	-			-	-		
20	*2*	*4.17/t 6.2.*	*65.6*	*CH2*	*2*	*4.12/t 6.2.*	*65.4*	*CH2*
21	*2*	*2.01/q 6.2*	*28.6*	*CH2*	*2*	*1.98/q 6.1*	*28.5*	*CH2*
22	*2*	*3.49/t,6.2*	*68.3*	*CH2*	*2*	*3.47/t,6.2*	*68.2*	*CH2*
23		-	-			-	-	
24	*3*	*3.27/s*	*58.0*	*CH3*	*3*	*3.26/s*	*57.9*	*CH3*

Note: C-11 & C-16 carbons were not observed due to poor relaxation.

*^{1}H and ^{13}C NMR assignment for **Impurity 8***

Position	^{1}H	*ppm/J*	^{13}C	*DEPT*
1	-	-	-	
2	-	-	*154.9*	
3	-	-	*119.6*	
4	-	-	*162.7*	
5	*1*	*6.95/d,5.6*	*106.3*	*CH*
6	*1*	*8.23/d,5.6*	*149.8*	*CH*
7	*2*	*4.69/s*	*36.2*	*CH2*
8	-	-	-	
9	-	-	*150.8*	*C=N*
10	-	-	-	
11	-	-	*141.9*	
12	*1*	*7.70/m*	*114.8*	*CH*
13	*1*	-	*141.9*	*CH*
14	*1*	*7.30/dd,8.3&1.4*	*126.1*	*CH*
15	*1*	*7.50/m*	*119.7*	*CH*
16	-	-	*141.9*	
17	-	-	-	
18	*3*	*2.24/s*	*10.4*	*CH3*
19	-	-	-	
20	*2*	*4.11/t 6.2.*	*65.1*	*CH2*
21	*2*	*1.98/q 6.2*	*28.7*	*CH2*
22	*2*	*3.47/t,6.2*	*68.3*	*CH2*
23	-	-	-	
24	*3*	*3.27/s*	*57.9*	*CH3*
25	-	-	-	
26	-	-	*153.9*	*C=N*
27	-	-	-	
28	-		*139.8*	
29	*1*	*7.10-7.14/m*	*121.6*	*CH*
30	*1*	*7.42/m*	*114.4*	*CH*
31	*1*	*7.42/m*	*114.4*	*CH*
32	*1*	*7.10-7.14/m*	*121.6*	*CH*
33	-	-	*139.8*	
34	-	-	-	

Formation of Impurities



^{1}H and ^{13}C NMR assignment for **Impurity 11**

Position	^{1}H	*ppm/J*	^{13}C	*DEPT*
1				
2			*152.0*	
3			*119.7*	
4			*163.3*	
5	*1*	*6.91/d,5.6*	*106.2*	*CH*
6	*1*	*8.18/d,5.6*	*147.2*	
7	*2*	*4.31&4.41/2d/12.9*	*38.5*	*CH2*
8				
9			*162.5*	*C=N*
10				
11			*146.3*	
12	*1*	*7.01-7.12/m*	*121.7*	*CH*
13	*1*	*7.46/m*	*112.8*	*CH*
14	*1*	*7.46/m*	*112.8*	*CH*
15	*1*	*7.01-7.12/m*	*121.7*	*CH*
16			*146.3*	
17				
18	*3*	*1.9/s*	*9.8*	*CH3*
19				
20	*2*	*4.09/m*	*65.0*	*CH2*
21	*2*	*1.98/m*	*28.6*	*CH2*
22	*2*	*3.35/m*	*68.2*	CH2
23				
24	*3*	*3.17/s*	*57.9*	*CH3*
25	*1*	*7.87/s*	*66.9*	*CH*
26				
27			*154.2*	*C=N*
28				
29			*143.3*	
30	*1*	*7.01-7.12/m*	*122.0*	*CH*
31	*1*	*7.27&7.43/m*	*117.9*	*CH*
32	*1*	*7.27&7.43/m*	*117.9*	*CH*
33	*1*	*7.01-7.12/m*	*122.0*	*CH*
34			*143.3*	
35	*1*	*12.53/bd*		*NH*
36			*152.4*	
37				
38		*8.46/d,5.6*	*147.7*	
39	*1*	*7.01-7.12/m*	*108.0*	*CH*
40			*163.3*	
41			*119.9*	
42				
43	*2*	*4.09/m*	*65.5*	*CH2*
44	*2*	*1.98/m*	*28.7*	*CH2*
45	*2*	*3.42/m*	*68.3*	CH2
46				
47	*3*	*3.13/s*	*58.0*	*CH3*
48	*3*	*1.99/s*	*10.2*	*CH3*

The positive electro spray ionization (ESI) mass spectrum showed the *m/z* peaks at 685 $[M+H]^{+}$, 686 is the isotopic peak of mass 685. IR spectrum displayed characteristic absorptions at 3435, 2924, 1600-1400 and 900-675 cm^{-1} which is indicative of NH stretching, asymmetric CH_2 stretching aromatic –C=C-, -C=N- stretching, and ether functionality which is supported by quaternary carbon signals in ^{13}C and DEPT spectrum. ^{13}C NMR is accounted for 36 carbons. DEPT spectrum displayed seven negative signals corresponds to seven methylene and thirteen positive signals corresponds to four methyl and thirteen methine groups in which eight of them appeared as four signals. Ten extra signals in ^{13}C NMR other than DEPT correspond to twelve quaternary carbons in which four of them appeared as two signals. A singlet at 7.87 ppm, one exchangeable proton in the molecule observed in D_2O exchange study and 2D NMR data confirms the substitution of Impurity 3 to the Impurity-3 at 25th position.

Based on the above spectral data the molecular formula of Impurity-11 could be $C_{36}H_{40}N_6O_4S_2$. This molecular formula matched well with the protonated molecular ion observed at *m/z* 685 in the mass spectral data. The above

data obtained from the spectral studies can be rationalized in terms of Impurity **11** is having the molecular formula $C_{36}H_{40}N_6O_4S_2$ and corresponding structure was characterized as 1-[[1H-benzo[d]imidazol-2-ylthio][4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylthio]-1H-benzo[d]imidazole. (RRT =4.81)

CONCLUSION

Information of the different possible impurities, metabolites, and their synthetic routes is a prerequisite for thorough understanding of impurity profile in the manufacturing of the anti-ulcerative drug rabeprazole. Keeping in view this regulatory requirement of rabeprazole impurities, the process related impurities and metabolites in rabeprazole bulk drug were identified, synthesized and characterized using mass, IR and NMR spectral data.

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