

Synthesis and Characterization of Process Related New Impurity in Ufiprazole

Dr. Sandip Sadaphal,* Huang Xiangliang, Rajesh Ambre, Sonal Chothe, Wei xiong and Xu cong

Menovo R & D centre, Ningbo Menovo pharmaceutical Co. Ltd., Shangyu, Zhejiang, CHINA

* Correspondence: E-mail: sandip@menovopharm.com

(Received 10 Dec, 2018; Accepted 11 Jan, 2019; Published 18 Jan, 2019)

ABSTRACT: Impurities identification, synthesis and its control are the key factor for drug quality and specification. Impurity study enhances scope towards better production of drug molecules. New impurities 5-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylthio)-1H-benzo[d]imidazole and 6-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylthio)-1H-benzo[d]imidazole have been identified, synthesized and analyzed in ufiprazole (5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylthio)-1H-benzo[d]imidazole). Both dimer impurities were isolated with HPLC purity more than 98% and characterized by IR, ¹H-NMR, ¹³C-NMR and LCMS.

Keywords: Ufiprazole; dimer impurities; synthesis; purification; identification and characterization.

INTRODUCTION: Active pharma ingredients Impurities impact on quality, yield, physical properties and safety of drug molecule.^{1,2} Currently in active pharma ingredients synthesis, impurities are the hot topic, as FDA has a close eye on quality of drug molecules. In the regulatory guidelines of the International Conference on Harmonization (ICH), it is recommended that impurities integrated to more than 0.1% should be identified and characterized.¹

In active pharma ingredients synthesis obtained product with 100% purity is very rare instead if it's in salt form. The main sources of organic impurities are key starting materials, used reagents, residual solvents, by-products and process degradation impurities. Generally impurities form during manufacturing process, during drying of substance or during stability or storage of product.

For any drug registration basic criteria for drug substance is to specify both known as well as unknown impurities present as per ICH guidelines Q3A(R), Q3B(R) and Q3C.³

The main challenge in front of researchers during synthesis of drug molecule is identification and preparation of impurities in pure form to make its standard sample. The prepared impurities can use for reference standard for analytical research and in validation study of drug molecules.

Ufiprazole^{4,5} (5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylthio)-1H-benzo[d]imidazole (1) is a key intermediate for omeprazole (2), esomeprazole potassium (3), esomeprazole sodium (4), esomeprazole magnesium dihydrate (5) and esomeprazole trihydrate (6) as shown in figure 1.

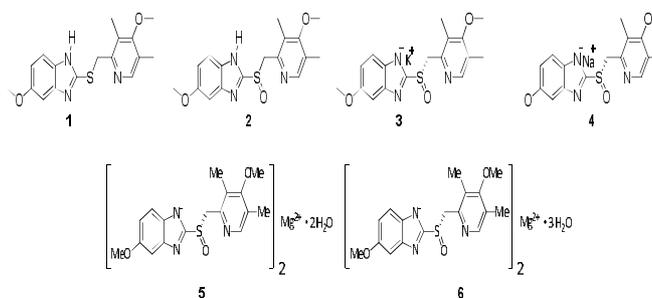


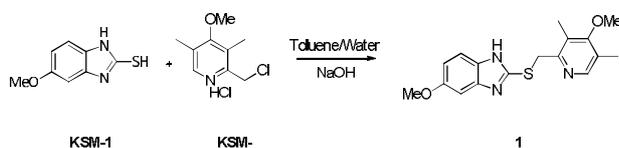
Figure 1: Chemical structures of ufiprazole, related intermediates and API's.

Omeprazole, chemically 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole and its therapeutic uses were disclosed in European Patent No. 5129. Omeprazole sold in market under the brand name Prilosec and Losec where as esomeprazole sold under the brand name Nexium. Both are a medication used in the treatment of gastroesophageal reflux disease, peptic ulcer disease, and Zollinger–Ellison syndrome. Omeprazole has a stereogenic center at sulfur atom and therefore exists as a mixture of two optical isomers such as R-

omeprazole and S-omeprazole (esomeprazole). These drugs are referred as proton pump inhibitors.⁶

By considering importance of ufiprazole in pharmaceutical products, its quality has on prime importance. Therefore, in depths study undertaken in this article, to identify, synthesize and separate pure impurities samples of ufiprazole. This study will help to understand the source, identification, synthesis, purification technique and characterization of dimer impurities in ufiprazole. It supports to get rid of unknown impurities or identified impurities.

RESULTS AND DISCUSSION: However, in literature there is no reference available to identify, synthesize and characterize the dimer impurities.⁴ This make extreme need to identify unknown impurities present in synthesized ufiprazole which is more than limit of ICH guidelines (NMT 0.1%). These unknown related impurities were identified, and their structures were tentatively assigned on the basis of their fragmentation patterns in LC-MS. In the present work, the identified impurities of ufiprazole were synthesized and characterized by various spectroscopic techniques and further confirmed by spiking studies using qualitative HPLC analysis. The following scheme represents synthesis of ufiprazole (**1**).



Scheme 1: Preparation of Ufiprazole (1). Conditions: NaOH, Toluene/water, 45-50°C, 18h, 80-85%.

The manufacturing process of ufiprazole start with key starting materials 2-mercapto-5-methoxybenzimidazole (KSM-1) and 2-(chloromethyl)-4-methoxy-3,5-dimethyl-4-methoxy pyridine hydrochloride (KSM-2) to prepare sulfide (**1**) by condensation reaction. During condensation reaction formation of two unknown impurities were observed (dimer impurities of ufiprazole, Figure 2).

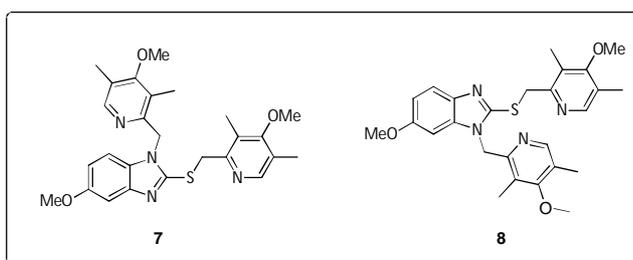


Figure 2: Dimer impurities in ufiprazole.

The observed two new impurities were identified by high performance liquid chromatography (HPLC, Figure 3). Impurities vary in between 0.03% to 0.5%. These unknown impurities molecular weight is obtained from liquid chromatographic mass spectrometry (LC-MS). After getting molecular weight in +ve mode we took efforts to predict two unknown impurities present in synthesized ufiprazole. After extensive study we identified impurities and synthesized it by chemically. Synthesized impurities were submitted for spectral analysis and identified. These two impurities have similar *R_f* values on TLC. Obtained two impurities were prepared and purified using solvent purification to get excellent purity (~98%). Using spectral analysis impurities were identified as mentioned in Fig. 2.

In obtained impurities 5-methoxy-1-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (**7**, Dimer-201) is available commercially, but another positional isomer 6-methoxy-1-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (**8**, Dimer-202) is yet unknown and reported it here first time. The following HPLC chromatogram has shown both dimer impurities (**7** and **8**) at RT 25.869 and 27.093 respectively and ufiprazole at RT 13.806.

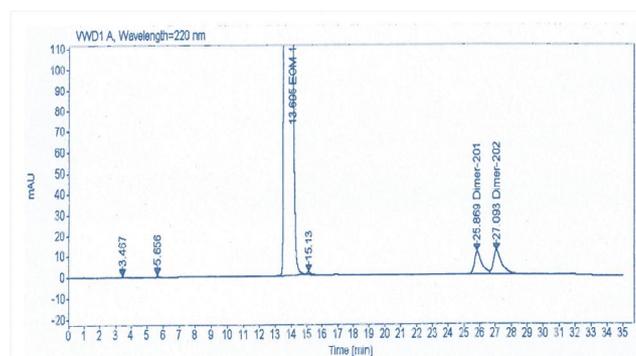
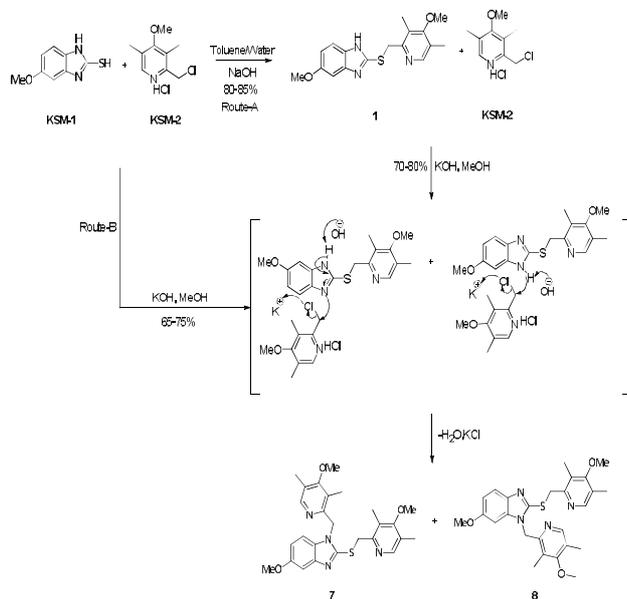


Figure 3: Characteristic HPLC chromatogram of ufiprazole with dimer impurities.

The 5-methoxy, 2-thiol-1H-benzimidazole (KSM-1) and 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (KSM-2) are reacted (coupling reaction) to yield 5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl) methyl)thio)-1H-benzo[d]imidazole (**1**). The reaction is driven by base, and must be conducted under basic biphasic condition. After completion, the reaction is worked up by phase separation, by washing the organic layer with water. The solvent is concentrated by distillation, and the product **1** is isolated by crystallization from toluene.

During formation of ufiprazole, formation of 5-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1*H*-benzo[*d*]imidazole (**7**, Dimer-201) and another positional isomer 6-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1*H*-benzo[*d*]imidazole (**8**, Dimer-202) were observed.



Scheme 2: Source of impurities of ufiprazole.

These identified dimer impurities can be prepared by using two routes as shown above in scheme 2. Compound **7** and **8** (dimer impurities) were prepared using both methods and isolated products have excellent purity after several solvent purifications.

Route A, firstly need to prepare compound **1** and then condense it with KSM-2 (1:1.5 eq.) in the presence of methanolic KOH solution at 45-50°C. Whereas, in route B, KSM-1 and KSM-2 (1:2.5 eq.) were used in the presence of methanolic KOH solution. Both methods lead to provide impurity **7** and **8** in 60:40% ratio on HPLC. By using column chromatography it's difficult to separate two positional isomer as their retention factor (*R_f*) is same on TLC. Therefore need to develop a purification method to separate two dimers with excellent quality.

Separation of impurities: To separate dimer impurities ethyl acetate was used as solvent for crystallization at 70-80°C with 30 volumes and age to 0-5°C for 2h after gradual cooling. The obtained ratio of impurities in solid and mother liquor is 85%: 66%. After successive 4-5 times purification of obtained impurities got pure dimer impurity **7** with purity 99%. To obtain another dimer impurity we have combined all mother

liquor obtain from purification of dimer impurity **7** and purified it with ethyl acetate 4-5 times to obtain another dimer impurity **8** with purity 98%. The isolation and purification details as shown below in Table 1.

Table 1: Purification details and obtained ratio of dimer impurities **7 and **8**.**

Compound	Purification	Solvent	Volume	Dimer impurity ratio (%)	
				Compound 7	Compound 8
Crude	NA	NA	NA	55±3	45±3
Solid	1st purification	Ethyl acetate	30	60±3	40±3
	2nd purification		30	78±3	25±3
	3rd purification		30	87±3	13±3
	4th purification		30	93±3	7±3
	5th purification		30	99	<1
Mother liquor	1st purification	Ethyl acetate	12	40±3	60±3
	2nd purification		12	35±3	65±3
	3rd purification		12	25±3	75±3
	4th purification		12	10±3	90±3
	5th purification		12	<2	98

The obtained mother liquors were distilled out using vacuum and used for purifications.

Using purification method to obtain different ratio of dimer impurities it may vary with experiments to experiments. After successfully separation of dimer impurities, analyzed with IR, ¹H NMR, ¹³C NMR, and LC-MS and their structures are confirmed.

The main difference observed in dimer impurities compound **7** and **8** is in ¹H NMR as shown below;

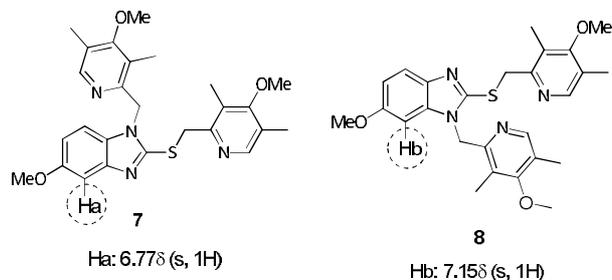


Figure 3: ¹H NMR values differentiation of proton Ha and Hb (6.77d and 7.15d respectively).

The proton Ha and Hb have different chemical shift values as environment of both protons are different. Hb is more de-shielded with respect to Ha due to more inductive effect of pyridine ring in **8**.

Experimental Section:

General : The chemicals and solvents were purchased from commercial suppliers and they were used as such. All IR spectra (KBr) were recorded on a Perkin-Elmer 1430 spectrometer, ¹H NMR and ¹³C NMR spectra were recorded on 400 MHz Bruker FT-NMR

spectrometers. All chemical shifts are given as δ value with reference to tetra methyl silane (TMS) as an internal standard. Mass spectra and high resolution mass spectrum were recorded on Agilent 6120B series single quadrupole LC-MS and Q-Tof micro YA019 instrument. All melting points were determined with the WRR visual melting point apparatus.

General procedure for the preparation of 5-methoxy-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (1): In water added NaOH (2.5eq) and cool to room temperature, then charge toluene (5V) and age for 10 mins. In water toluene solution added 2-mercapto-5-methoxybenzimidazole (1.04 eq.) then charge 2-chloromethyl-3,5-dimethyl-4-methoxy pyridine hydrochloride (1.0 eq.) at 20-25°C and stir for 10-15 mins under nitrogen purging. Raise temperature to 45-50°C to get clear solution and maintain for 4-5h. Check HPLC of organic layer to confirm consumption of starting material and formation of product. After completion of reaction separate out organic layer and wash with water (2.5V). Obtained organic layer distilled out up to 50% and then cool to 0-5°C for 12h. Filter the reaction mass at 0-5°C under Nitrogen atmosphere. Wash with toluene (1V) at 0-5°C. Filtered solid dry at 45-50°C to get ufiprazole as white solid (80-85%), HPLC purity 99%; m.p. 122 - 126°C. IR (KBr): 3072, 2995, 1635, 1481, 1156 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): $\square\square$ 12.48 (s, 1H, exchangeable with D₂O), 8.15 (s, 1H), 7.39 (s, 1H), 6.93 (s, 1H), 6.74 (dd, $J = 2.12, 8.68$ Hz, 1H), 6.73 (s, 1H), 4.65 (s, 2H), 3.75 (s, 3H), 3.68 (s, 3H), 2.25 (s, 3H), 2.16 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO- d_6): $\square\square$ 163.9, 155.7, 154.0, 149.1, 149.1, 125.6, 125.0, 118.3, 111.0, 110.5, 101.1, 94.9, 60.2, 55.9, 37.0, 13.3, 11.3 ppm; LC-MS (ESI⁺): Calcd m/z for C₁₇H₂₀N₃O₂S [M+H]⁺: 330, found: 330

5-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (7): 5-methoxy, 2-thiol-1H-benzimidazole (1.0eq.) and 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (2.5 eq.) were reacted in presence of KOH (3.6 eq., maintain reaction pH ~12-14) in methanol (10V) at 45-50°C for 18-24h. After completion of reaction (monitored on HPLC) distilled out methanol completely to get solid residue. Obtained solid was charged in water (10V) and age for 1h at room temperature. Filter obtained solid and suck dry. Wet cake dissolved in ethyl acetate (30V) at 75-80°C under stirring. After get clear solution, cool gradually to room temperature and then cool to 0-5°C for 2h. Filter to obtained solid and repeat same crystallization pro-

cess 4-5 times to get white colored solid dimer impurity (7) with HPLC purity 99%; m.p.:156-159°C; IR (KBr): 3056, 2988, 1621, 1489, 1125 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): $\square\square$ 8.11 (s, 1H), 7.97 (s, 1H), 7.47 (d, $J = 8.72$ Hz, 1H), 6.91 (d, $J = 2.12$ Hz, 1H), 6.76 (dd, $J = 2.28, 8.72$ Hz, 1H, 1H), 5.38 (s, 2H), 4.57 (s, 2H), 3.70 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 2.25 (s, 3H), 2.18 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO- d_6): $\square\square\square$ 163.7, 163.7, 155.9, 154.4, 153.2, 150.6, 149.0, 148.9, 137.9, 125.5, 125.3, 124.9, 123.8, 118.5, 110.5, 94.5, 60.2, 60.2, 55.9, 46.7, 38.2, 13.3, 13.2, 11.3, 10.5 ppm; LC-MS (ESI⁺): Calcd m/z for C₂₆H₃₁N₄O₃S [M+H]⁺: 479, found: 479.

6-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole(8): To obtain Dimer-202 impurity used mother liquor obtained from 5-methoxy-1-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-2-(((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (7) impurity. All obtained mother liquors combined together and distilled out at 40-45°C under reduced pressure to get solid residue. Obtained residue check with HPLC (ratio of dimer 201 and dimer 202) and then purified with ethyl acetate (12V) at 75-80°C and then cool to 0-5°C for 2-4h. filtered solid and again purified with ethyl acetate 4-5 times to get desired dimer 2 impurity as a white solid with HPLC purity 98%; m.p.:136-139°C; IR (KBr): 3000, 2964, 1623, 1489, 1144 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): $\square\square$ 8.13 (s, 1H), 7.98 (s, 1H), 7.17 (s, 1H), 7.15 (s, 1H), 6.73 (dd, $J = 2.4, 8.68$ Hz, 1H), 5.38 (s, 2H), 4.64 (s, 2H), 3.77 (s, 3H), 3.71 (s, 3H), 3.69 (s, 3H), 2.24 (s, 3H), 2.22 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO- d_6): $\square\square\square$ 163.8, 163.7, 155.7, 154.4, 153.2, 152.2, 149.1, 149.0, 144.1, 131.8, 125.6, 125.0, 124.0, 111.1, 110.6, 101.3, 60.3, 60.2, 55.9, 47.0, 38.0, 13.3, 13.3, 11.3, 10.6 ppm; LC-MS (ESI⁺): Calcd m/z for C₂₆H₃₁N₄O₃S [M+H]⁺: 480, found: 480.

CONCLUSION: We have successfully identified synthesized and analyzed process impurities present in ufiprazole. This is the first report of dimer impurities of ufiprazole. Ufiprazole intermediates used in the synthesis of omeprazole, esomeprazole and related salts. The data reported in this article will help to identify and make specifications of unknown impurities in ufiprazole and their related compounds.

ACKNOWLEDGEMENT: Author thanks Menovo pharmaceutical Co. Ltd. management for their support and encouragement.

REFERENCES:

1. International Conference on Harmonization (ICH) guidelines. Q3A (R) impurities in New Drug Substances. ICH guidelines: Geneva, Switzerland, February, 2002.
2. Szekely, G.; Amores de Sousa, M.C.; Gil, M.; Castelo Ferreira, F.; Heggie, W. Genotoxic impurities in pharmaceutical manufacturing: Sources, regulations, and mitigation. *Chem. Rev.* 2015, *115*, 8182.
3. ICH Q3A (R2) Guideline, Impurities in New Drug Substances (version 4); 2006.
4. Kuraray Co. Ltd. Patent: US6350876 B2, 2002 ; Reddy, R.; Upparapalli, S.; Sivadas, A.; Vigneswara, R.; shanmuga, B.; subbaiah, R., patent: WO2011/12957 a1, 2011 and Cipla limited; curtis, Philip, Anthony Patent: WO2008/102145 A2, 2008.
5. Fryklund, J.; Wallmark, B.; *Journal of Pharmacology and Experimental Therapeutics* 1986, *236* (1), 248.
6. Lindberg, P.; *Med. Res. Rev.*, 1990,*10*,1.