Identification, Synthesis and characterization of Retigabine dimer impurities

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Abstract:

In the course of the process development of Retigabine, two possible potential impurities were observed by simple reverse phase high-performance liquid chromatography (HPLC). Primarily, the two impurities were identified by the liquid chromatography–mass spectrometry (LC–MS) data followed by synthesized and Characterized these two impurities as dimers of Retigabine. Based on the spectral data (^{1}H NMR, ^{13}CNMR, IR and MS), the structure of Impurity-1 and Impurity-2 were characterized as diethyl (4,4'-diamino-6,6'-bis((4-fluorobenzyl)amino)-[1,1'-biphenyl]-3,3'-diyl)dicarbamate and diethyl (methylenebis(6-amino-4-((4-fluorobenzyl)amino)-3,1-phenylene))dicarbamate.

Introduction:

Retigabine [1], is an anticonvulsant and used as treatment for partial epilepsies, with the chemical name of ethyl N-[2-amino-4-[(4-fluorophenyl)methylamino]phenyl]carbamate. The drug was developed by Valeant Pharmaceuticals and GlaxoSmithKline. It was approved by the European Medicines Agency under the trade name Trobalt on March 28, 2011, and by the United States Food and Drug Administration (FDA), under the trade name Potiga, on June 10, 2011. It works primarily as a potassium channel opener that is, by activating a certain family of voltage-gated potassium channels in the brain.

The existence of impurities or it's related compounds in a drug substance can have a major impact on the quality of the drug product. During the process development of Retigabine, impurities were observed not more than 0.15% level along with the main product peak in the HPLC analysis. As per the guidelines suggested by the ICH to qualify the drug substance, the amount of acceptable level for a known and unknown related compound (impurity) should be less than 0.15 and 0.10%, respectively. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized.

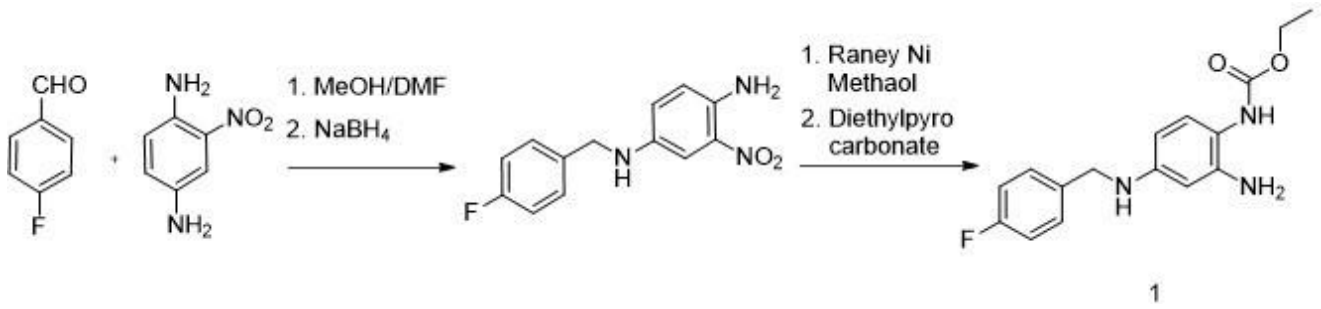
Hence, a comprehensive study was undertaken to identify, synthesize and characterize these two impurities of Retigabine. In this article, we report the synthesis, isolation and spectral characterization of impurities obtained during the process development of Retigabine.

Results and discussion:

Synthesis of Impurity-1 and Impurity-2:

During the process development of Retigabine, two potential impurities were detected by simple reverse phase high-performance liquid chromatography (HPLC). Firstly, the impurities were identified by the liquid chromatography–mass spectrometry (LC–MS) data. Based the LC-MS data these two impurities were identified as Retigabine dimer (Impurity-1) and Retigabine methylene dimer (Impurity-2).

Scheme 1. Synthesis of Retigabine



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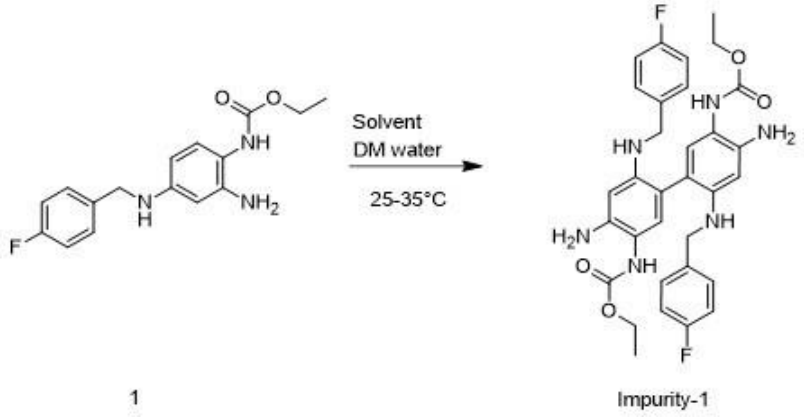
As mentioned in the scheme 1, synthetic process of Retigabine contains Methanol as a solvent. An in-house HPLC method is developed to separate the related substances from Retigabine, and this method contains Acetonitrile solvent as Diluent and Mobile phase. In presence of Methanol and Acetonitrile solvents, these two impurities formation was observed.

Synthesis of Impurity-1:

Impurity-1 (Retigabine dimer) is forming in presence of Methanol or Acetonitrile solvent as a degradation impurity. Impurity-1 was enriched to 30% by dissolving the Retigabine in excess methanol and maintaining at room temperature for 20 days, further purified through preparative HPLC method.

Scheme 2. Synthetic scheme for impurity-1.



1 Impurity-1

The following experiments were conducted to enrich the level of Impurity-1 (Retigabine dimer) impurity by dissolving the Retigabine in different solvent conditions.

Table 1. Experimental conditions and results.

S. No.	Solvent + Water	Solvent + Water Ratio	Maintenance Time	Impurity-1 (%)
1	Acetonitrile + water	9:1	6 days	0.08
2	Acetonitrile + water	1:1	6 days	2.25
3	Acetonitrile + water	1:2	8 days	9.34
4	Acetonitrile + water (PH -5 with HCl)	1:1	8 days	14.74
5	Acetonitrile + water (PH -8 with NaOH)	1:1	8 days	0.06
6	Acetone + water	3:8	8 days	6.63
7	THF + water	1:2	8 days	9.22
8	Methanol + water	1:1	8 days	11.8
9	Methanol + water	1:1	20 days	30.63

Among the all solvents, in methanol and water with the ratio of 1:1 formation of dimer impurities enriched to 30.6% (impurity-2). Further purified the Impurity-2 (Retigabine dimer) by using preparative HPLC.

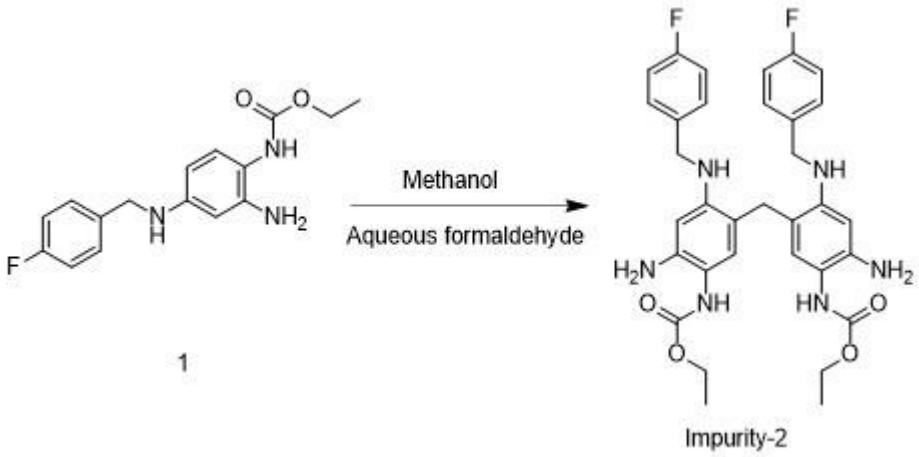
Experimental condition:

To a stirred solution of Methanol (2500 mL) and DM water (2500 mL) Retigabine (5.0 g) was added and stirred for 8 days. Precipitated solid (Impurity-1) was filtered and stirred filtered solution for 22 days (impurity-1 enriched to 30.6%). Solvent evaporated under vacuum and purified the crude compound using preparative HPLC offered Impurity-1 (Retigabine dimer) (0.4 g).

Synthesis of Impurity-2:

Impurity-2 (Retigabine Methylene dimer) is forming due to presence of traces formaldehyde as contaminant in Methanol and Acetonitrile. Hence, Impurity-2 was synthesized by adding formaldehyde to Retigabine in methanol solvent.

Scheme 3. Synthetic scheme for Impurity-2.

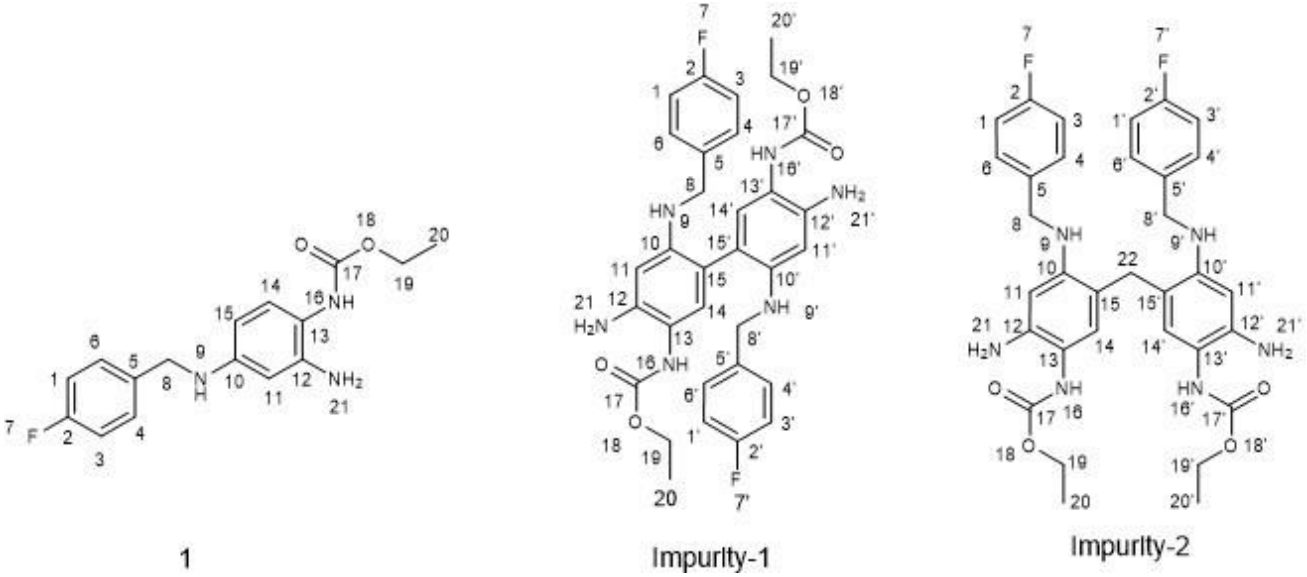


Experimental condition:

To a stirred solution of Retigabine (1.0 g, 3.3 mmol) in 500 mL of methanol, 500 mL of DM water and aqueous formaldehyde was added (3.3 mmol) and the reaction mixture was stirred for 5 h at room temperature. The precipitated solid was filtered and dried under vacuum to offered Impurity-1 in 80.5% of yield as off-white solid.

Structure Elucidation (Charecterization) of Retigabine and its impurities:

Fig 1. Structures of Retigabine and Retigabine impurities with position numbers for the atoms.



Structure elucidation of Retigabine

ESI mass spectrum of Retigabine exhibited molecular ion peak at *m/z 304* [M+1] in positive ion mode, indicating the mass of Retigabine to be 303. In ^{1}H NMR of Retigabine, the signals at 7.36ppm (2 aromatic), 7.14 ppm (2 aromatic), 6.69 ppm (1 aromatic), 5.83 ppm (1 aromatic), 5.92 ppm (1 aromatic), 4.16 (CH_2), 4.04 (CH_2), 1.18 (CH_3) were observed. In IR spectrum, 3421 (1^o amine N-H stretching), 3375 (2^o amine N-H stretching), 3037 (aromatic C-H stretching), 2985 (aliphatic C-H stretching), 1701 (C=O stretching), 1522 (C=C stretching), 1068 (O-C stretching), 1601 (1^o amine N-H bending) bands were appeared. The ^{13}C NMR spectrum displayed signals due to 16 carbons and the DEPT spectrum displayed two negative signals due to the presence of two –CH2 groups and seven positive signals, due to presence of seven -CH groups. Based on the above spectral data the molecular formula of Retigabine was confirmed as $C_{16}H_{18}FN_3O_2$ and the corresponding structure was confirmed as ethyl(2-amino-4-((4-fluorobenzyl)amino)phenyl)carbamate.

Table 2
^{1}H NMR (in DMSO-d_6), ^{13}C NMR, DEPT data (in CD_3OD) of Retigabine.

Position	**Retigabine**			
	^{1}H	**ppm/*J***	**^{13}C**	**DEPT**
1	1H	7.36-7.33/m	116.29	CH
2	-	-	164.9	-
3	1H	7.36-7.33/m	116.5	CH
4	1H	7.14-7.08/m	130.58	CH
5	-	-	138.01	-
6	1H	7.14-7.08/m	130.49	CH
7	-	-	-	-
8	2H	4.16-4.15/d	50.13	CH_2
9	1H(NH)	5.89-5.86/t	-	-
10	-	-	149.86	-
11	1H	5.92-5.91/d-2.5	129.24	CH
12	-	-	138.04	-
13	-	-	115.76	-
14	1H	6.69-6.67/d-7.4	102.45	CH
15	1H	5.83-5.80/dd-8.3, 2.4	106.03	CH
16	1H(NH)	8.13/s	-	-
17	-	-	162.49	-
18	-	-	-	-
19	2H	4.04-3.99/q	62.56	CH_2
20	3H	1.18	15.45	CH_3
21	2H(NH_2)	4.51/s	-	-

Structure elucidation of Impurity-1 (Retigabine dimer)

ESI mass spectrum of impurity-1 exhibited the protonated molecular ion peak at *m/z 605* [M+1] in positive ion mode, indicating the molecular weight of this impurity to be 604. In 1H NMR spectrum, the doublet of doublet signal at 5.83 - 5.80 ppm (*j* = 8.3, 2.4 Hz, 1H) assigned to 15th position proton of Retigabine was disappeared in impurity-1, further more two doublet signal assigned to 11th and 14th protons are appeared as two singlet signals and rest of the chemical shift values are similar to that of Retigabine. This evident suggested that 15th position of Retigabine is involved in carbon-carbon bond formation between two molecules and its molecular mass is matched well with the molecular mass obtained in ESI mass spectrum, and this was supported by DEPT spectrum by displayed quaternary carbon signal at 122.1 ppm instead of methine carbon signal at 106.3 ppm. Based on the above spectral data, the molecular formula of impurity-1 could be $C_{32}H_{34}F_2N_6O_4$ and the corresponding structure was characterized as diethyl(4,4'-diamino-6,6'-bis((4-fluorobenzyl)amino)-[1,1'-biphenyl]-3,3'-diyl)dicarbamate.

Structure elucidation of Impurity-2

ESI mass spectrum of impurity-2 exhibited molecular ion peak at *m/z 619* [M+1] in positive ion mode, indicating the molecular weight of this impurity to be 618 which is 14 amu greater than impurity-1. In ^{1}H NMR spectrum, the chemical shift values are similar to that of impurity-1, except a new singlet signal at 3.44 ppm with two proton integration. This information suggested that, methylene group was flanked by two molecules of Retigabine at 15th position and this was supported in DEPT spectrum by displayed negative signal at 33.08 ppm. Based on the above spectral data, the molecular formula of impurity-2 could be $C_{33}H_{36}F_2N_6O_4$ and the corresponding structure was characterized as diethyl(methylenebis(2-amino-4-((4-fluorobenzyl)amino)-5,1-phenylene))dicarbamate.

Table 3

^{1}H NMR (in DMSO-d_6), ^{13}C NMR, DEPT data (in CD_3OD) of impurity-1 and impurity-2.

Position	Impurity-1				Impurity-2			
	^{1}H	ppm/Multiplicity	^{13}C	DEPT	^{1}H	ppm/Multiplicity	^{13}C	DEPT
1,1'	2H	7.32-7.28/m	116.58	CH	2H	7.20-7.16/m	116.57	CH
2,2'	-	-	165.1	-	-	-	165.03	-
3,3'	2H	7.32-7.28/m	116.79	CH	2H	7.20-7.16/m	116.78	CH
4,4'	2H	7.10-7.06/m	130.68	CH	2H	7.00-6.96/m	130.37	CH
5,5'	-	-	136.42	-	-	-	136.73	-
6,6'	2H	7.10-7.06/m	130.6	CH	2H	7.00-6.96/m	130.29	CH
7,7'	-	-	-	-	-	-	-	-
8,8'	4H	4.26-4.17/m	50.14	CH_2	4H	4.14/s	50.13	CH_2
9,9'	2H(NH)	-	-	-	2H(NH)	-	-	-
10,10'	-	-	158.63	-	-	-	158.29	-
11,11'	2H	6.18/s	131.2	CH	2H	5.85/s	129.15	CH
12,12'	-	-	145.99	-	-	-	146.84	-
13,13'	-	-	136.21	-	-	-	136.7	-
14,14'	2H	6.79/s	120.14	CH	2H	6.53/s	121.18	CH
15,15'	-	-	122.1	-	-	-	124.78	-
16,16'	2H(NH)	8.62/brs	-	-	2H(NH)	-	-	-
17,17'	-	-	162.68	-	-	-	-	-
18,18'	-	-	-	-	-	-	-	-
19,19'	4H	4.09-4.04/q	63.18	CH_2	4H	3.97-3.96/m	63.28	CH_2
20,20'	6H	1.23-1.20/t	15.36	CH_3	6H	1.14	15.31	CH_3
21,21'	4H(NH_2)	-	-	-	4H(NH_2)	-	-	-
22	-	-	-	-	2H	3.44/s	33.08	CH_2

Table 4

Mass, FT-IR spectral data and retention time in HPLC for Retigabine, impurity-1 and impurity-2

S.No.	Compound	RT	Mass	IR
1	Retigabine	25.32	304	3421 (1^o amine N-H stretching), 3375 (2^o amine N-H stretching), 3037 (aromatic C-H stretching), 2985 (aliphatic C-H stretching), 1701 (C=O stretching), 1522 (C=C stretching), 1068 (O-C stretching), 1601 (1^o amine N-H bending).
2	Impurity-1	39.9	605	3564 (1^o amine N-H stretching), 3393 (2^o amine N-H stretching), 2983 (aromatic C-H stretching), 2930 (aliphatic C-H stretching), 1682 (C=O stretching), 1510 (C=C stretching), 1061 (O-C stretching), 1606 (1^o amine N-H bending).
3	Impurity-2	40.46	619	3350 (1^o amine N-H stretching), 3290 (2^o amine N-H stretching), 3040 (aromatic C-H stretching), 2983 (aliphatic C-H stretching), 1683 (C=O stretching), 1532 (C=C stretching), 1061 (O-C stretching), 1603 (1^o amine N-H bending).

Conclusion:

In conclusion, we have identified the process related impurities of Retigabine. These impurities were synthesized and characterized as diethyl (4,4'-diamino-6,6'-bis((4-fluorobenzyl)amino)-[1,1'-biphenyl]-3,3'-diyl)dicarbamate (Impurity-1 / Retigabine dimer) and diethyl (methylenebis(6-amino-4-((4-fluorobenzyl)amino)-3,1-phenylene))dicarbamate (Impurity-2 / Retigabine methylene dimer).

Acknowledgments

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Reference:

1. WO 2012 098075 A1.
2. Xiangjun Wang, Hui Zhou, Jianbin Zheng, Chao Huang, Weixia Liu, Lushan Yu, Su Zeng, Journal of Pharmaceutical and Biomedical Analysis, 2012, 71, 148-151.
3. ICH Guideline Q3A (R), Impurities in New Drug Substances, February 7, 2002.
4. Michael Salzinger and Johannes A. Lercher, Green Chem., 2011, 13, 149–155.

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