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SYNTHESIS AND CHARACTERIZATION OF POTENTIAL IMPURITIES OF DEFERASIROX

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ABSTRACT
Deferasirox **(1)** is an orally active iron chelating agent, invented by Novartis AG. During process development for deferasirox, we observed four related substances (impurities) namely deferasirox methyl ester **(2)**, deferasirox ethyl ester **(3)**, bis(salicyl)imide compound **(4)** and cyclic poly ester compound **(5)**. The present work describes synthesis, characterization and analytical condition for detection and purity of these impurities.

KEYWORDS
Deferasirox, Iron (Fe^{+3}) chelating agent, related substance, Impurities

INTRODUCTION
Deferasirox is an iron chelating compound and is used in the treatment of patients suffering from chronic iron overload due to blood transfusion of greater than twenty units. Deferasirox is commercially available under the brand name of EXJADE and supplied as a dispersible tablet with different strengths.

The excess of iron (metal) deposited in body tissues can cause severe damage to organs such as liver, the heart and the endocrine organs and can lead to death. Iron chelators are able to excrete the iron deposited in the organs and thus, lower the iron related morbidity and mortality. Deferasirox is one of such iron chelating agent, designated chemically as 4-[3,5-bis(2-hydroxy phenyl)-1H-1,2,4-triazol-1-yl]benzoic acid. It is a tridentate ligand and binds iron with high affinity i.e. 2:1 ratio.

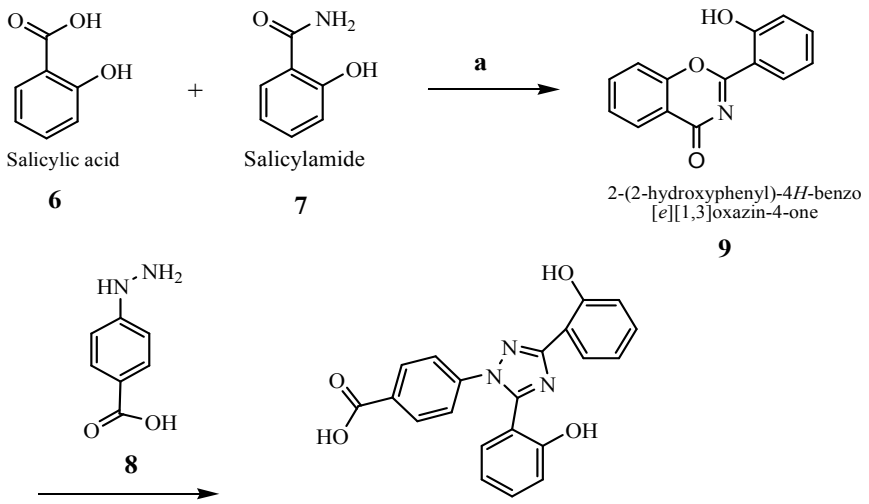
The presence of impurities: in an active pharmaceutical ingredient (API) can impact the quality and safety of the drug products. International Conference on Harmonization (ICH) guidelines recommends identifying and characterizing all impurities present in an API at a level of < 0.10 % [I]. This limit is calculated based on maximum daily dosage. However, in deferasirox, the limit is further tightened to $\leq$ 0.05 % because of the high maximum daily dosage (i.e.,~2.4 g). These impurities are required in pure form to check the analytical performance characteristics such as specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, system suitability testing and relative retention factor [II]. In our current

work, we identified four related substances in deferasirox API. Their detection, origin, synthesis and characterization are described in this article.

Following are the four related substances identified in our deferasirox **(1),** Methyl 4-[3,5-bis(2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]benzoate (deferasirox methyl ester) **(2)** Ethyl 4-[3,5-bis(2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]benzoate (deferasirox ethyl ester) **(3)**, 2-hydroxy-N-(2-hydroxybenzoyl)benzamide [bis(salicyl)imide compound] **(4)** and poly ester compound **(5)**.

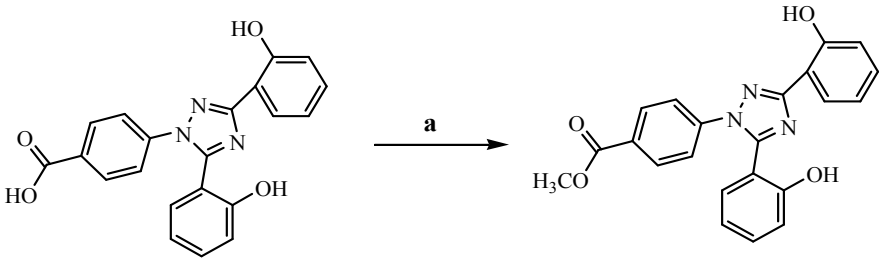
RESULTS AND DISCUSSION

Deferasirox **(1)** was synthesized by the known literature synthetic procedure [3-6] (Scheme 1). A key intermediate, 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one **(9)**, was obtained by reacting salicylic acid **(6)** with salicylamide **(7)** in o-xylene and thionyl chloride in presence of catalytic amount of pyridine [III-VII]. Further, condensation of compound (**9)** with 4-Hydrazino-benzoic acid **(8)** in boiling methanol results desired Deferasirox **(1)** [VIII].



Scheme 1. Synthetic scheme of deferasirox **1**. reagents and conditions: (a) o-xylene, Thionyl chloride, pyridine, 140-145^{0}C, 2h, ethanol, Yield = 72-77%; (b) methanol or ethanol, 60-80^{0}C, 70 min, Yield = 92-95%.

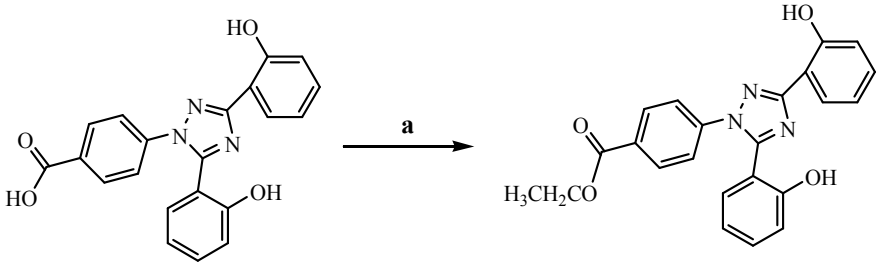
Deferasirox methyl ester **(2)** formed during the preparation of deferasirox in boiling methanol [IX] with 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one **(9)** and 4-Hydrazino-benzoic acid **(8)** to form **(2)** as an impurity**.** Related substance **(2)** was prepared by reported reference in literature [X].



1 **2**

Scheme 2. Synthetic scheme of deferasirox methyl ester **2**. Reagents and conditions: (a) methanol, concentrated H_2SO_4, reflux, 20 hrs, Yield = 87%

Deferasirox ethyl ester (**3)** is formed as an impurity in the synthesis of deferasirox. Ethyl 4-Hydrazino benzoate [XI], which is a contaminant in 4-hydrazino benzoic acid (**8)**, further, reacts with 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one (**9)** to produce deferasirox ethyl ester (**3)**. Related substance (**3)** was prepared from deferasirox in boiling ethanol in presence of concentrated sulfuric acid (scheme 3). The mass spectrum showed a molecular ion at m/z 402.3 [$(M+H^+)$], and the infrared (IR) spectrum displayed carbonyl stretching at 1715.9 cm^{-1}. In view of deferasirox, we observed an additional triplet at δ 1.35 – 1.39 ppm (3H) and quartet at δ 4.35 – 4.40 ppm (2H) in the ^1H NMR and in ^{13}C NMR characteristic signal at δ 14.07 ppm and 61.04 ppm corresponding to additional ethyl groups. In ^{13}C NMR, considerable change in chemical shift of carbonyl group from 166.4 ppm to 164.8 ppm indicates ester formation.

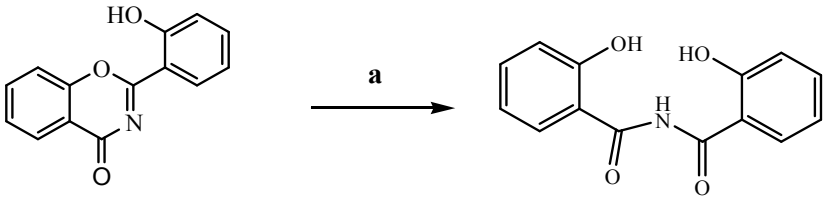


1 **3**

Scheme 3. Synthetic scheme of deferasirox ethyl ester **3**. Reagents and conditions: (a) Ethanol, concentrated H_2SO_4, reflux, 14 hrs, Yield = 78%

Bis(salicyl)imide (**4**), [XII, XIII] is the degradation product of 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one (**9)**, in acidic/basic medium at room temperature. If any unreacted compound **(9)** is presence in deferasirox API on storage, then it is converted into the compound (**4)**. This related substance (**4)** was prepared by reaction of compound **(9)** with water and methanol as a solvent (scheme 4).

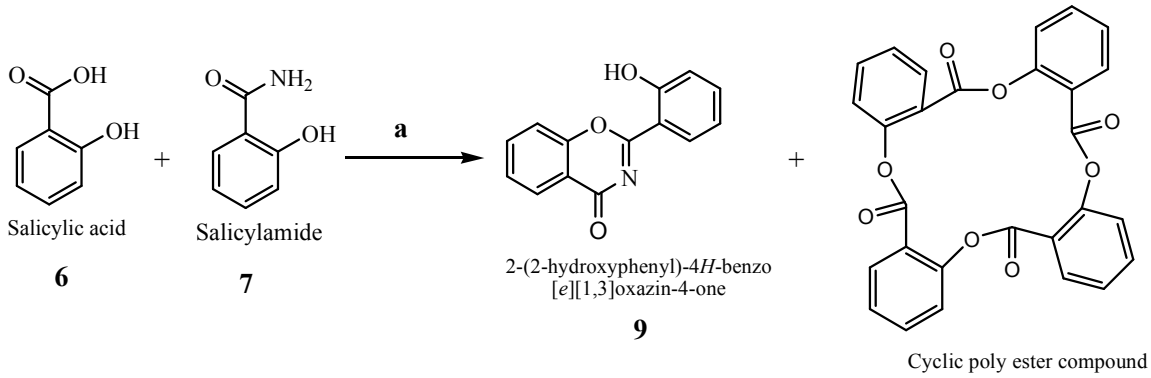
The mass spectrum of compound (**4)** showed molecular ion peak at m/z 256.07 [$(M-H)^-$]. The ^1H NMR spectrum showed peak at δ 11.46 ppm (2H, 2 x OH). This was confirmed by D_2O exchange. IR spectrum of related substance (**4)** displayed carbonyl group stretching at l70l.0 cm^{-1} and N-H and O-H group stretching at 3247.7 cm^{-1}. This compound (**4)** was spiked with deferasirox sample containing compound (**4)** and confirmed the related substance.



9 **4**

Scheme 4. Synthetic scheme of bis(salicyl)imide **5**. Reagents and conditions: (a) methanol, water, 50-55^{0}C, 10 hrs, Yield = 93%.

Cyclic poly ester compound (**5)** is formed as an impurity during synthesis of 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one **(9)**. The origin of cyclic poly ester compound **(5)** shown in Scheme 5.



Scheme 5. Synthetic scheme of Cyclic poly ester compound **6**. reagents and conditions: (a) o-xylene, Thionyl chloride, pyridine, 140-145^{0}C, 2h, ethanol, Yield = 15%.

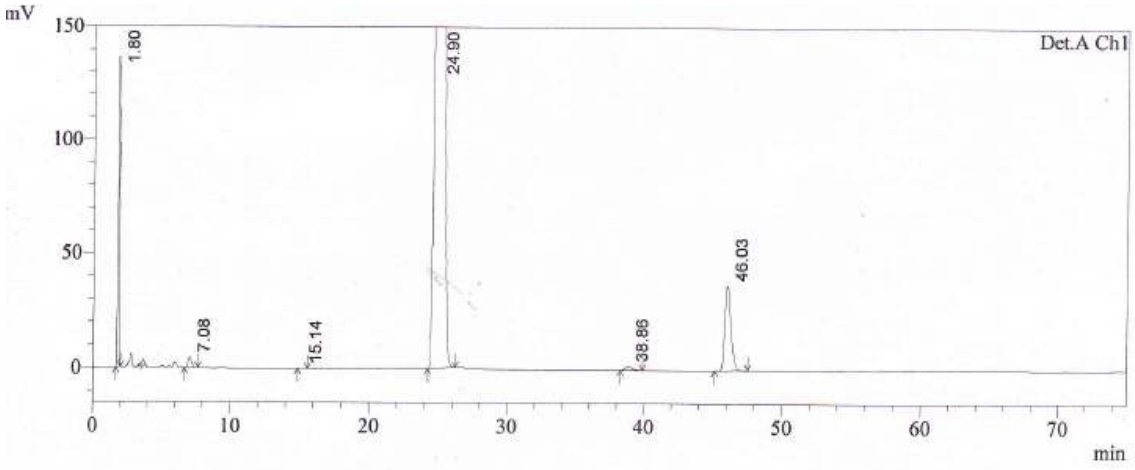
This related substance was prepared by reported reference in literature [XIV].

All observed related substance was analyzed by following analytical conditions:
Mobile Phase Preparation:
Buffer Preparation:
Dissolve 1.36 gm of potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled water and adjust the pH 2.0 with ortho phosphoric acid.
Organic mixture: Acetonitrile:methanol (80:20)
Mobile Phase A: Buffer
Mobile Phase B: Organic mixture
Diluent-1: Acetonitrile: methanol (50:50)
Diluent-2: Acetonitrile: Water (70:30)

Gradient Program:

Time(Min)	Mobile Phase-A (%)	Mobile Phase-B (%)
0.01	60	40
35.0	45	55
55.0	30	70
65.0	25	75
66.0	60	40
75.0	60	40

System	HPLC Shimadzu LC 2010
Column	250mm X 4.6 mm X 5µ packing L1(Kromasil C8)
Flow rate	1.4 ml/Min
Detector	UV / PDA detector
Wavelength	247 nm
Temperature	35^0C
Injection Volume	50 µl
Run Time	75 minutes
Auxiliary Range	1.0
Sample cooler	10^0C
Retention time of deferasirox	About 22 to 25 minutes
Relative retention time	2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one**(10)**: 0.61 Deferasirox Methyl ester **(2)** : 1.56 Deferasirox Ethyl Ester **(3)** : 1.85 Bis(Salicyl)imide **(4)** : 0.28 4-Hydrazino benzoic acid **(8)** : 0.07



Results

Detector A Ch1 247nm

Peak#	Name	Ret. Time	Area	Area %	RRT
1	4-Hydrazino benzoic acid	1.80	1073164	3.155	0.07
2	Bis(salicyl)imide	7.08	83302	0.251	0.28
3	2-(2-hydroxy phenyl)-4H-1,3-benzoxazin-4-one	15.14	911	0.003	0.61
4	Deferasirox	24.90	31692807	93.160	1.00
5	Deferasirox methyl ester	38.86	56698	0.167	1.56
6	Deferasirox ethyl ester	46.03	1111014	3.266	1.85
Total			34019897	100.000	

CONCLUSION

To have a thorough understanding of impurity formation for origin of iron chelating agent deferasirox, it is essential to have detailed information about various potential impurities and their synthetic routes. In view of regulatory importance of the impurities in the API, a detailed study on various impurities in deferasirox was conducted. Different process-related impurities and degradation products in deferasirox were identified. They were synthesized and characterized using various spectroscopic techniques such as ^{1}H- NMR, ^{13}C-NMR, and IR. This characterization was supported by liquid chromatography-mass spectrometry (LC-MS) and mass spectral data.

EXPERIMENTAL SECTION

Solvents and reagents were obtained from commercial sources and used without purification. The IR spectra (ν max cm^{-1}) were recorded in solid-state KBr dispersion using a Shimadzu (IR Prestige-21) Fourier transform (FT)-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400-MHz spectrometer. The chemical shifts were reported in δ ppm relative to tetramethylsilane (TMS). The mass spectra were recorded on a API 2000 Perkin-Elmer PE-SCIEX mass spectrometer. Melting points were determined on a VEEGO melting-point apparatus (model no. VMP-D).

Preparation of Ethyl 4-[3,5-Bis(2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]benzoate (3)

Deferasirox **(1)** (10.0 g, 0.0267 mol) was added to Ethanol (50 ml) followed by addition of concentrate sulfuric acid (10 ml) at 25-30^{0}C and refluxed for 14 hrs. Check TLC [Mobile Phase: Toluene: Ethyl acetate: Acetic acid]. After completion of reaction distil out ethanol completely

under vacuum at 50-55^{0}C. Charge 60 ml methylene dichloride in residue at 25-30^{0}C and wash this methylene dichloride layer with 3 x 100 ml water. Organic layer dried over sodium sulphate and distil out methylene dichloride completely under vacuum at 40-45^{0}C to give compound **(3)** (8.4 gm, Yield = 78.14 %); M.P.: 146-150^{0}C; IR (KBr pellet), cm^{-1}: 3275.1 (O-H, str.), 3059.4 (aromatic C-H, str.), 2987.4, 2904.6 (aliphatic C-H, Str.), 1715.8 (C=O, str.), 1622.7, 1607.6, 1586.7, l5l5.1 (C=N & C=C, str.), 1254.5 (C-N, str.), 772.5 and 752.1 (aryl CH out-of-plane bend); ^{1}H NMR (400MHz, DMSO-d_6): δ 1.32 (t, 3H, CH_3 of ethyl ester, J = 7.12), 4.33 (q, 2H, CH_2 of ethyl ester, J=7.12), 6.86 (d, 1H, J= 8.02), 7.02 (m, 3H), 7.39 (m, 2H), 7.39 (m, 2H), 7.56 (d, 1H, J = 8.78), 7.59 (d, 2H, J = 8.50), 8.05 (d, 1H, 7.68), 10.03 and 10.79 (2 brs, 2 x OH); ^{13}C NMR (75MHz, DMSO-d6): δ 14.1, 61.0, 113.6, 114.4, 116.1, 117.0, 119.5, 119.7, 123.4, 126.8, 129.5, 130.1, 131.0, 131.5, 132.6, 141.4, 152.1, 155.1, 156.3, 159.9, 164.8; MS m/z : 402.1 [$(M+H)^+$]. Anal. calcd. for $C_{23}H_{19}N_3O_4$ (401.4): C, 68.82; H, 4.76; N, 10.41. Found; C, 68.76; H, 4.71; N, 10.41. HPLC retention time ~ 47.6 min (RRT ~ 1.68).

Preparation of 2-Hydroxy-N-(2-hydroxybenzoyl)benzamide (4)

2-(2-Hydroxyphenyl)-4H-1,3-benzoxazin-4-one **(9)** (10.0 g, 0.042 mol) was added to mixture of methanol (50 ml) and water (50 ml). Reaction mass heated slowly to 50-55^{0}C and maintain for 8-10 h. Check for TLC against compound **(9) [**Toluene 5 ml: Ethyl acetate 5 ml]. After completion of reaction cool reaction mass to 25-35^{0}C and stir for more 1 h. Filter the resulting slurry and dry material at 50-55^{0}C for 8-10 h to give product **(4)** as off-white crystals (10 gm, Yield = 93.02%); M.P.: 195-200^{0}C; IR (KBr pellet), cm^{-1} : 3247.7 (N-H, O-H, str.), 1700 (C=O, Str.), 1608, 1592.8 (aromatic C=C, Str.), 1249 (C-N, Str.), 754.6, 745.6 (aromatic CH out-of-plane bend); ^{1}H NMR (400 MHz, DMSO-d6): δ 6.96-7.05 (m, 2 X 2H, O- and P-position of phenol), 7.47 (dd, 2 X 1H, J = 7.96 Hz, 1.90 Hz, m-phenol), 7.84 (d, 2 X 1H, J = 7.96 Hz), 11.36 (brs, 2 X OH), 12.00 (brs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d6): δ 117.1, 119.0, 119.7, 130.6, 134.3, 156.9, 164.5; MS m/z : 255.9 [$(M - H)^-$]. Anal. Calcd. for $C_{14}H_{11}NO_4$ (257.2): C, 65.37; H, 4.30, N, 5.44. Found: C, 65.29; H, 4.23; N, 5.41. HPLC retention time ~ 14 min (RRT ~ 0.60).

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