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Degradation Impurities in Linagliptin Drug Product

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A B S T R A C T

Three degradation impurities related to Linagliptin (**1**) drug product, *N*-Acetyl Linagliptin (**2**), *N*-Aminoacyl Linagliptin (**3**) and *N*-Formyl Linagliptin (**4**) have been synthetically prepared and characterized. These impurities form due to interaction of excipient and active pharmaceutical ingredient (API) during formulation. Formation of above drug product degradation impurities has been investigated.

Keywords: Linagliptin, excipient, drug product, degradation, impurities

A R T I C L E I N F O

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1. Introduction

Degradation products in drug product formulations are sought to be tightly controlled by pharmaceutical industries. Depending upon the drug's maximum daily dose, regulatory guidelines require the characterization and toxicological evaluation of degradation impurities. Drug degradation of products is generated by interaction of many

excipient and active pharmaceutical ingredients (API) [1-2]. Many of the API's involves hydrolysis, oxidation or specific interaction of drugs with reactive impurities in excipient [3-5]. Understanding the excipient (reactive impurity sources) and its impact on the drug product stability is essential in designing and developing a robust drug product [6-7]. As a part of ongoing research on Linagliptin (**1**) drug product formulation, we envisaged formation of three degradation impurities, namely *N*-Acetyl Linagliptin (**2**), *N*-Aminoacyl Linagliptin (**3**) and *N*-Formyl Linagliptin (**4**).

2. Experimental

Reagents

Acetyl chloride, triethyl amine, acetic anhydride, Formic acid and urea were used as raw materials. Linagliptin used for the preparation of impurities was prepared as per the literature reported method. Dichloromethane, hexanes and ethanol were used as solvents to carry out the reaction and for crystallization of the product. All the above solvents were purified by the reported procedures [8].

Instrumentation

Melting points are measured in Polmon MP96 capillary melting point apparatus and are uncorrected. The ^{1}H NMR spectra was recorded in Varian 500 MHz FT NMR spectrometer in DMSO-d_6. The chemical shifts were reported in ppm relative to TMS (δ 0.00 ppm) as internal standard. IR spectra were recorded on a Perkin-Elmer FT-IR instrument (KBr pellet method). Mass spectra were recorded using a 4000-Q-trap LC-MS/MS mass spectrometer. The solvents and reagents were used without further purification. Chromatographic purity of impurities was analyzed qualitatively by HPLC.

Experimental procedure (Material Synthesis)

Preparation of (*R*)-*N*-[1-[7-(But-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl]piperidine-3-yl]acetamide [*N*-Acetyl Linagliptin] **(2):** Linagliptin (2 g, 0.004 mole) was dissolved in methylene chloride (20 ml) at 20-30°C. It was cooled to 0-5°C and triethylamine (0.65 g, 0.006 mol) was added slowly over a period of 15 min. To the above stirred solution, acetyl chloride (0.4 g, 0.005 mol) was added slowly over a period of 10 min at 0-5°C. The reaction mass was stirred at 0-5°C for 1 h and quenched by adding ice-cold water (20 ml). Temperature was raised to room temperature. The organic layer was separated and washed with aqueous sodium chloride solution (20 ml, ~10% w/w). The washed methylene chloride layer was concentrated at 40-45°C to obtain the product.

The concentrated mass was crystallized from hexanes and filtered. It was dried at 40-45°C under reduced pressure of ~20 mm Hg. Yield: 2.2g. A pale yellow powder (Hexanes); M.P.: 208-209°C; IR (KBr): 3690, 2831, 2410, 2126, 1550, 1265 cm^{-1}; ^{1}H NMR (500 MHz, *CDCl$_3$*): ¢ (*ppm*); 1.71-1.88 (*m*, 4*H*), 1.80 (*s*, 3*H*), 2.02 (*s*, 3*H*), 2.88 (*s*, 3*H*), 3.31-3.73 (*m*, 4*H*), 3.74 (*s*, 3*H*), 4.17 (*m*, 1*H*), 4.85 & 4.94 (*ABq*, 2*H*), 5.56 (*s*, 2*H*), 6.77 (*d*, 1*H*), 7.52 (*dd*, 1*H*), 7.76 (*dd*, 1*H*), 7.87 (*d*, 1*H*), 8.01 (*d*, 1*H*); Mass (PE SCIEX-API 2000) ESI *in +ve ion mode*: m/z; 515 [M+H]$^+$.

Preparation of (*R*)-*N*-[1-[7-(But-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-2, 6-dioxo-2, 3, 6, 7-tetrahydro-1*H*-purin-8-yl] piperidine-3-yl] urea

[*N*-Aminoacyl Linagliptin] **(3):** Linagliptin (10g, 0.021 mol) was suspended in DM water (200 ml). Urea (12.8 g, 0.213 mol) was added along with tetrabutylammonium bromide (0.2 g, catalytic) and the reaction mixture was heated to 90-100°C. Nitrogen gas was bobbled through the reaction mass. The progress of the reaction was monitored by HPLC. After completion of the reaction, it was cooled and the product was filtered out. The wet product was crystallized from hot ethanol (50 ml) to obtain pure *N*-Aminoacyl Linagliptin. The product was dried at 40-45°C under reduced pressure of ~20 mm Hg till constant weight. Yield: 5 g. A white powder (Ethanol); M.P.: 230-234°C; IR (KBr): 3447, 2911, 2266, 1999, 1407, 954 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): ¢ (*ppm*); 1.39-1.85 (*m*, 4*H*), 1.76 (*s*, 3*H*), 2.88 (s, 3*H*), 2.91-3.69 (*m*, 4*H*), 3.40 (*s*, 3*H*), 3.56 (*m*, 1*H*), 4.90 (*s*, 2*H*), 5.31 (*s*, 2*H*), 5.46 (*brs*, 2*H*), 6.10 (*d*, 1*H*), 7.67 (*dd*, 1*H*), 7.81 (*d*, 1*H*), 7.91 (*dd*, 1*H*), 8.24 (*d*, 1*H*). Mass (PE SCIEX-API 2000) ESI *in +ve ion mode*: m/z; 516 [M+H]$^+$.

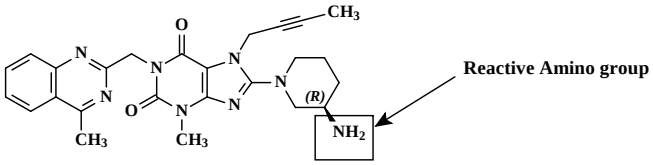
Preparation of (*R*)-*N*-[1-[7-(But-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl]piperidine-3-yl]formamide

[*N*-Formyl Linagliptin] **(4):** Formic acid (5.85 g, 0.127 mol) was added slowly to acetic anhydride (10.8 g, 0.105 mol) at 20-30°C slowly over a period of ~30 min under nitrogen atmosphere. The mixture was heated to 40-45°C and stirred for 1h. Thereafter, it was cooled to 18-20°C and Linagliptin (5 g, 0.010 mol) was added. The reaction mass was stirred at room temperature for one hour. The progress of the reaction was monitored by checking TLC. After completion of the reaction, it was quenched by adding 100 ml of DM water. It was stirred for 20-30 min at room temperature. Ethyl acetate (50 ml) was added to it and stirred for another 20 min during which product was precipitated out. It was filtered and washed with water (25 ml). The product was dried at 40-45°C under reduced pressure of ~20 mm Hg till constant weight. Yield: 4 g**.**

Light brown powder (ethyl acetate); M.P.: 176-179°C; IR (KBr): 3265, 2941, 1699, 1661, 1567, 1519 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): ¢ (*ppm*); 1.73-1.86 (*m*, 4*H*), 1.79 (*s*, 3*H*), 2.88 (*s*, 3*H*), 2.89-3.54 (*m*, 4*H*), 3.55 (*s*, 3*H*), 4.26 (*m*, 1*H*), 4.82 and 4.94 (*ABq*, 2*H*), 5.55 (*s*, 2*H*), 6.93 (*d*, 1*H*), 7.51 (*t*, 1*H*), 7.75 (*t*, 1*H*), 7.85 (*d*, 1*H*), 8.0 (*d*, 1*H*), 8.18 (*s*, 1*H*). Mass (PE SCIEX-API 2000) ESI *in +ve ion mode*: m/z; 501 [M+H]$^+$.

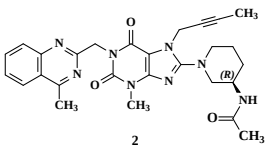
3. Results and Discussion

Linagliptin (**1**), trade names *Tradjenta* (US) and *Trajenta* (worldwide) is a DPP-4 inhibitor developed by *Boehringer Ingelheim* for treatment of type II diabetes. Linagliptin (once-daily) was approved by the U.S. Food and Drug Administration (FDA) on 2 May 2011 for treatment of type II diabetes [9]. Linagliptin drug substance (**1**), having the following chemical structure contains a reactive primary amino group.



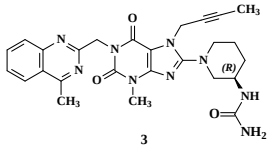
8-[(3R)-3-amino-1-piperidinyl]-7-(but-2-yn-1-yl)-methyl-1-[(4-methylquinazolin-2-yl) methyl]-3, 7-dihydro-1H-purine-2,6-dione [Linagliptin]

Three of the major degradation impurities which need to be monitored in Linagliptin drug product are *N*-Acetyl Linagliptin (**2**), *N*-Aminoacyl Linagliptin (**3**) and *N*-Formyl Linagliptin (**4**), having following chemical structures.



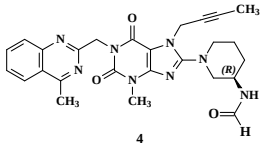
2

***N*-ACETYL LINAGLIPTIN**



3

***N*-AMINOACYL LINAGLIPTIN**



4

***N*-FORMYL LINAGLIPTIN**

***N*-Acetyl Linagliptin (2):**

Many often a time, acetic acid is generated due to degradation of excipient used during formulation process of APIs. Acetic acid, which generates due to degradation of excipient, may undergo nucleophilic attack by reactive primary amino group present in Linagliptin, resulting in the formation of *N*-Acetyl Linagliptin impurity (**2**).

To prepare authentic *N*-Acetyl Linagliptin impurity, an independent synthesis was carried out by reacting Linagliptin drug substance with acetyl chloride in the presence of triethyl amine as base (scheme 1). This reaction yielded *N*-Acetyl Linagliptin almost quantitatively. *N*-Acetyl Linagliptin (**2**) prepared through above process is characterized by ^{1}H NMR, IR and Mass spectroscopy. The detail of these characterization data for *N*-Acetyl Linagliptin (**2**) has been given in the experimental section.

***N*-Aminoacyl Linagliptin (3)**:

Quite frequently, formulation of API's is carried out using starch containing urea as one of the excipient. (*R*)-1-Methyl-1-[3-phenyl-3-(*o*-tolyloxy) propyl] urea [atomoxetine *N*-amide] impurity, which forms due to interaction of excipient with Atomoxetine drug substance, *is* listed in Atomoxetine hydrochloride USP capsule monograph as one of the degradation product [10]. In a similar way, urea present in excipient may react with primary amino group of Linagliptin giving rise to *N*-Aminoacyl Linagliptin impurity (**3**).

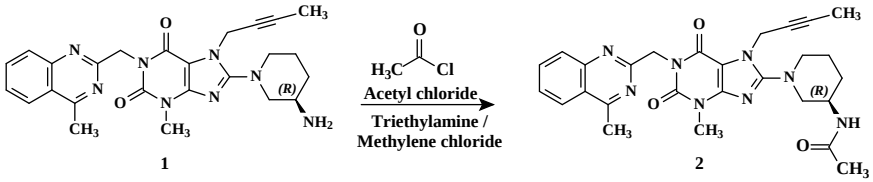
N-Aminoacyl Linagliptin (**3**) was prepared synthetically by reacting Linagliptin with urea in water at higher temperature, as shown in scheme 2. *N*-Aminoacyl Linagliptin prepared through above process is characterized by ^{1}H NMR, IR and Mass spectroscopy. The detail of these characterization data for *N*-Aminoacyl Linagliptin has been given in the experimental section.

***N*-Formyl Linagliptin (4):**

Formaldehyde is known degradant of many excipients like polyethylene glycol and polysorbates. Formaldehyde is susceptible to air oxidation and could be partially converted to formic acid. Therefore, excipients having residual formaldehyde are expected to contain some formic acid impurity as well.

Formic acid, which generates due to degradation of excipient, may undergo nucleophilic attack by primary amino group present in Linagliptin, resulting in the formation of *N*-Formyl Linagliptin impurity (**4**), as shown below. *N*-Formyl Linagliptin (**4**) was prepared synthetically by reacting Linagliptin with a mixture of acetic anhydride and formic acid, as shown in the below synthetic scheme 3.

N-Formyl Linagliptin (**4**) prepared through above process is characterized by ^{1}H NMR, IR and Mass spectroscopy. The detail of these characterization data for *N*-Formyl Linagliptin has been given in the experimental section.



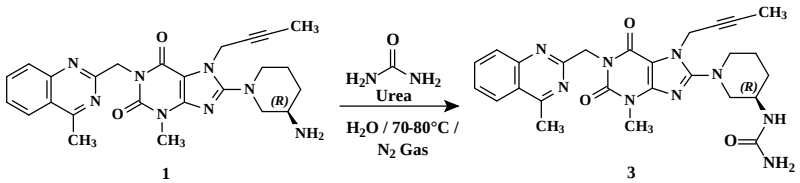
1

LINAGLIPTIN

2

***N*-ACETYL LINAGLIPTIN**

Scheme 1: Synthesis of N-Acetyl Linagliptin



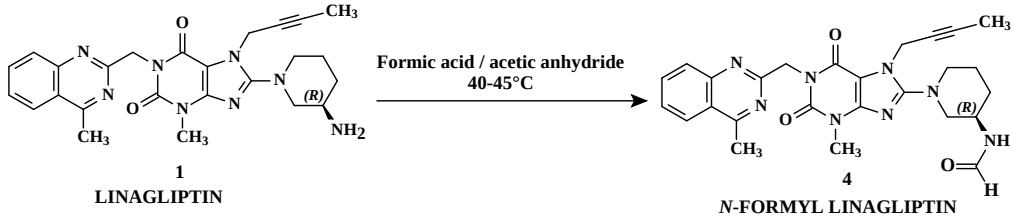
1

LINAGLIPTIN

3

***N*-AMINOACYL LINAGLIPTIN**

Scheme 2: Synthesis of *N*-Aminoacyl Linagliptin



1
LINAGLIPTIN

4
***N*-FORMYL LINAGLIPTIN**

Scheme 3: Synthesis of N-Formyl Linagliptin

4. Conclusion

Reactive impurities in pharmaceutical excipient could cause significant degradation of Linagliptin drug product. In this article, three probable Linagliptin degradation impurities have been discussed. Chemical synthesis of these degradation impurities has been reported. These impurities are of great importance to monitor their presence during formulation process as well as during storage of Linagliptin drug product.

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6. References

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